INCO : International Scientific Cooperation Projects

Contract number : ICA4-CT-2001-10006

Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa.

First annual report: covering period 1/11/2001 to 31/10/2002

Acronym: COWIDI

Author: Coordonator principal CIRAD : D. BIEYSSE

Key words: *Fusarium xylarioides*, coffee wilt disease, *Coffea canephora*, genetic resistance.

Report n° 08/2003
CIRAD/AMIS
Crop Protection Program

CONTENTS

Contract number : ICA4-CT-2001-10006

COWIDI

Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa.

	NAMES	ORGANISATION / ADDRESS	TEL/FAX/E-MAIL
CIRAD Coordinator	Bieysse Daniel Plant Pathologist	CIRAD-AMIS TA40/02 Boulevard de la Lironde 34 398 Montpellier, Cedex 5, France	Tel : (33) 4 67 61 44 68 Fax : (33) 4 67 61 57 93 e-mail : bieysse@cirad.fr
	Pierre Charmetant Coffee Breeder	CIRAD-Cp. TA80/PS1 Boulevard de la Lironde 34 398 Montpellier, Cedex 5, France	Tel : (33) 4 67 61 71 01 Fax : (33) 4 67 61 59 83 e-mail : charmetant@cirad.fr
	Fabrice Pinard Plant Pathologist	CIRAD-CABI C/o CABI P.O BOX 633 Village Market, Nairobi – Kenya	Tel : 254 (2) 52 44 62 e-mail : f.pinard@cgiar.org
UCL	Henri Maraite	Université catholique de Louvain Unité de Phytopathologie Croix du Sud 2bte 3 B-1348 Louvain la Neuve - Belgium	Tel: 33 10 473749 Fax: 33 10 478697 e-mail: maraite@fymy.ucl.ac.be
CORI	Denis T. Kyetere Director of Research Plant Breeder	Coffee Research Institute P.O. Box 185, Mukono – Uganda	Tel : 256 077 700725 (Office) Mobile : 075 692 994 Fax : 04 1 32 10 70 e-mail : cori@africaonline.co.ug
	Georgina J. Hakiza Plant Pathologist	Coffee Research Institute P.O. Box 185, Mukono – Uganda	Tel : 256 077 700 725 (Office) Mobile : 075 692 994 (Office) e-mail : cori@africaonline.co.ug
	Pascal Musoli Breeder	Coffee Research Institute P.O. Box 185, Mukono – Uganda	Tel : 256 077 700 725 (Office) Mobile : 077 576 446 e-mail : cori@africaonline.co.ug
	Pauline Aluka. Breeder	Coffee Research Institute P.O. Box 185, Mukono – Uganda	Tel : 256 077 700 725 (Office) e-mail : cori@africaonline.co.ug
UNIKIN	Kalonji-Mbuyi	University de Kinshasa B.P. 866, Kinshasa XI RDC	Tel : (243) 99 88 873 E-mail : adrienkalonji@hotmail.com
	Patrick Tshilenge Djim	University de Kinshasa B.P. 866, Kinshasa XI RDC	Tel : (243) 98 24 95 82 e-mail : pdtshilenge@hotmail.com
CABI	Mike Rutheford	CABI Bioscience (CAB International) UK Centre, Bakeham Lane, Egham, Surrey, TW20 9TY, UK	Tel : 01491 – 82 90 70 Fax : 01491 – 82 91 00 e-mail : m.rutherford@cabi.org
	Julie Flood	CABI Bioscience (UK) Bakeham Lane Egham, Surrey – UK	Tel : + 44 1491 829000 Fax : + 44 1491 829100 e-mail :j.flood@cabi.org

SUMMARY

Coffee Wilt Disease due to *Fusarium xylarioides* is currently a major problem especially for Robusta coffee in DRC and Uganda. This vascular disease induces the death of the coffee tree within 9 to 15 months, depending on the susceptibility and on the agro ecological conditions. Replanting is impossible, due to the soil being infested for several years. Genetic resistance seems the only solution to fight the disease; indeed it is investigated by the COWIDI INCO DEV Project. The objectives of the Project are:

1- Study of the genetic diversity of the pathogen
2- Characterisation of the host/pathogen interaction
3- Identification of tolerant/resistant varieties, analysis of inheritance, elaboration of a breeding strategy.
4- Factors influencing the development of the disease

In order to study the genetic diversity of the pathogen, a collect of strains has been realised by CORI and CIRAD in the representative coffee areas in Uganda, and a collection has been initiated by CABI with a central data base regrouping baseline information on each isolate. A preliminary analysis of molecular variability using variable number tandem repeat (VNTR) has commenced. Initials tests give rise to almost identical fingerprint profiles for all strains. However ISSR PCR primers demonstrate some level of intraspecific variability. A conventional genetic approach involving assessment of Vegetative Compatibility Groups has been initiated by CABI. The sexual life cycle is in progress in UCL.

To study the aggressiveness and of the host pathogen/interaction, a standardised inoculation method by syringe injection of a calibrated suspension of conidia in the stem of young plantlets 8 to 10 months old is in progress at CORI, CIRAD, and UNIKIN. A protocol is experimented with a statistic design. Specific techniques adapted to the local constraints are also experimented by every partners. A ring test to compare results will be developed during the next year.

The first results at CIRAD show a variation in the aggressiveness of the isolates. These results permitted to earmark tester isolates for routine tests in CIRAD (CAB003). Work at CORI is in progress.

CIRAD made a first series of tests to evaluate the susceptibility of *Coffea canephora*. Representatives of the five genetic groups of the species expressed various levels of resistance with isolate CAB003. Populations 1160, 1173, 1219, and 1230 look resistant (To be confirmed). For some populations the response is clear, namely 1213, 1153, 1191, 1236 (susceptible). For others the rate is intermediate, around 50%. These results can be considered as a trend.

At CORI, clones and local germplasm have exhibited field resistance and will be included in a hybridisation scheme. Collection of seeds from genotypes representative of the available germplasm and rooting of cuttings has started. An MTA (Material Transfer Agreement) has been signed between CORI and partners via CIRAD.

At UNIKIN field evaluation in Yangambi Station is in progress. The 7 Robusta clones (L36, L48, L93, L147, L215, L251, and SA158) currently released to the farmers in Orientale, Nord- and Sud- Kivu Provinces, and the clones recommended for the Bas Congo region (LAF93, LAF159, S9, S19, S23) will be dispatched to the partners.

In DRC the survey in Bas Congo, Bandundu and Kasai Provinces indicated that no case of the disease could be find in any of the sites visited. An epidemiological survey is planned in Northern and North Eastern Province for December 2002.

In Uganda, a base line survey conducted between March and May 2002, established that, in the districts of Mukono, Bundibugyo, Rukungiri,Kayunga, Kibale, Hoima, Kiboga, Mubende, Luwero, Wakiso, Mpigi, there are coffee farms which are nearly 100% devastated

In Uganda, four sites in small holders farms, have been selected for epidemiological observations in Mayuge and Iganga districts (Eastern Uganda) where disease pressure is low. For comparison purpose, four sites are selected in Masakai and Rakai districts. Disease recording is planned every 4 weeks.
Multi location trials with tolerant and resistant varieties obtained from cuttings of different varieties of Robusta survivors of artificial inoculations have been planted in field trials at Kituza, and 10 selected Arabusta clones were also planted in comparative trials in wilt infected gardens at CORI, Mubende and Luwero districts.

These first results, together with the setting of various breeding trials, are the first steps towards further work in the future.

COWIDI Scientific annual report

Introduction

Coffee Wilt Disease due to *Fusarium xylarioides* is currently a major problem especially for Robusta coffee. The disease is only know in some African countries but it is a threat to other producing countries.
Sanitation practices such as uprooting the affected trees, burning them on the farm then replanting after sterilising the soil is presently the only way to fight the disease. It is expensive and time consuming, and it seriously affects the profitability of the coffee.
Breeding for new varieties combined with integrated disease management towards an effective prevention and control of outbreaks of the disease would bring a response to this threat to the countries' economy and to the survival of millions of coffee farmers.
The main objective of this project is to develop a global strategy to fight the disease, based on durable tolerance/resistance and adapted to the smallholders' agro-systems and to the conditions prevailing in Africa.

To reach this objective, specific and technological approaches has been developped during the first year.

1-Collection of isolates, study of the genetic diversity of the pathogen,).
2-Characterisation of host/pathogen relation (tests, cyto-histology).
3-Identification of tolerant/resistant varieties, analysis of inheritance, definition of a breeding strategy.
4-Factors influencing the development of the disease

WP1: Pathogen diversity

Task 1 (CORI-UNIKIN with participation of CIRAD-CABI):Survey and collection of anamorph and teleomorph forms of the fungus on various parts of the trees, possibly on alternative hosts, in infested regions. Maintenance and dispatch to European labs.

Plant material, namely pieces of stem tissue and branches, has been collected from coffee trees exhibiting symptoms of wilt disease. In Uganda, branches and stem pieces (15-20 cm in length) were collected from farmers' fields and also from 10 *Coffea canephora (*robusta coffee) and *C. excelsa* genotypes held at the hybrid trial sites at Kituza and at the germplasm collection at Kawanda Agricultural Research Institute rerspectively.

No collections have been made in the Democratic Republic of Congo (DRC) as yet. However, this will be possible through epidemiological surveys in Northern and North Eastern DRC planned for the near future.

To date, isolations of *F. xylarioides* from plant material have been made in the country of origin of the material, and forwarded to CABI and CIRAD. However, where appropriate and particularly where perithecia are observed, wood samples will be forwarded to UCL for investigations of the sexual stage of the fungus and isolation of *F. xylarioides*. Where possible, the collection, isolation and purification protocol developed during the inaugural project workshop held in Kampala (February 2002) was followed.

Task 2: (CABI) Identification, storage, and exchange of isolates. Use a designed facility to ensure the successful maintenance of the pathogen strains. All data used to characterise the strains to be maintained on a database.

To date, more than 150 isolates of *F. xylarioides* have been made available for the work package research, of which more than 100 are now being held at CABI Bioscience (CABI). Those held at CABI have either been donated from culture collections held elsewhere, isolated from coffee plant material as part of previous wilt investigations undertaken at CABI or freshly isolated from plant material under this project and as described under task 1 above.

All isolates received at CABI have already been, or will be, purified by single sporing and will be also confirmed as *F. xylarioides* by CABI mycologists prior to analysis proceeding. After quotation by CABI the isolates will be, redistributed isolates as appropriate to other European partners to facilitate their research but, given quarantine restrictions and the potential threat to coffee production. CABI will not provide isolates to partners in Africa other than those whose return to their country of origin is permitted by the agricultural authorities.

All purified (single spored) isolates of *F. xylarioides* are being maintained on Synthetic Nutrient Agar (SNA) medium for short-term storage at CABI and assigned with an unique accession number (or 'IMI' number), e'g' IMI23456. Isolates will also be freeze dried and/or placed under liquid nitrogen and deposited in the CABI Genetic Resources Collection for safe, longer term storage.

Development of an electronic database, based on 'Access' software, to store key information relating to the *F. xylarioides* isolates obtained for the work package research has been initiated. Ultimately all baseline information on each isolate, such as geographic origin and host type, type of analyses undertaken and results of key analyses will be inputted and may be accessed by all partners involved in the work package.

Task 3 (CORI-UNIKIN CIRAD-UCL): Evaluation of the variability in isolate aggressiveness using standard inoculation tests (WP1).

Six *C. canephora* (robusta) clones, 1s/2, 1s/3, 1s/6, 223/32, 257/35 and 258/24(0), were recommended by the Ugandan government as replacements for the existing planting material in Uganda prior to the coffee wilt outbreak in the early 1990s. Tests undertaken in 1997 (involving artificial inoculation of pot grown coffee plantlets with *F. xylarioides* under screenhouse conditions) showed that all six clones developed symptoms of the disease but that the level of susceptibility varied. Similar reactions were observed under field conditions. This research is being followed-up as part of the current project. Plantlets of these clones currently being raised in the nursery at Kituza will be inoculated with *F. xylarioides* by various techniques, including stem injection and root dipping, to determine which method is most effective and consistent with regard to symptom development. These trials will begin in November 2002.

Preliminary results obtained at Cirad are still to be confirmed. At this stage the isolates tested show all levels of aggressiveness.

Task 4: (CIRAD, CORI, UNIKIN UCL) Description of the fungal life cycle, asexual and sexual phases.

Activities relating to task 4 will commence in 2003. This work will focus on development of a model system to investigate the sexual cycle of *F. xylarioides*, analysis of character segregation in the sexual cycle and an assessment of the likelihood of isolates capable of overcoming arabusta clones arising through the sexual cycle. Based on VCG tester strains developed at CABI, vegetative compatibility between progenies of isolate crosses produced in mating tests will also be assessed.

Task 5 (CIRAD-CABI): Evaluation of the genetic diversity using a range of techniques including PCR and microsatellites.

In CABI For preliminary analysis of molecular variability and VCG, a representative subgroup of 15 isolates from Uganda and DRC (see Annex 2) has been selected. Extraction of DNA from these isolates, using a modified CTAB technique developed by Cubero *et al.* (1999)*, has yielded high quality DNA from each isolate. All DNA samples were amplifiable by PCR. Analysis of molecular variability using variable number tandem repeat (VNTR) primers and digestion of the rDNA IGS has commenced. Results of initial tests showed that amplification of DNA by VNTR primers RY (CAG^5] and ERIC 2 (enterobacterial repetitive intergenic consensus) primers each gave rise to almost identical fingerprint profiles for all strains. However, when amplified using five separate inter-simple-sequence-repeat-anchored (ISSR) PCR primers, AAC, ACA, CCA, CGA & TGT, primer CGA revealed no varition between the strains but each of the remaining primers demonstrated some level of intraspecific variability. ISSR is considered to be a useful genotyping tool for assessing variability across the entire genome (Taylor et al., 1999*). Data from this preliminary work will undergo full analysis shortly. Follow-on studies, now being initiated, will involve further amplification by ISSR primers in addition to single-enzyme (agarose gel-based) and dual-enzyme (acrylamide gel-based) amplified fragment length polymorphism (AFLP) analyses.

The molecular analyses are being supported by a conventional genetic approach involving assessment of vegetative compatibility between isolates. Mutant forms of the wild type isolates, that are unable to utilise nitrate and are required for subsequent pairing and vegetative compatibility group (VCG) identification, are also being generated and typed at CABI at present. Similar studies will be undertaken on *F. xylarioides* isolates originating from DRC during a training of a Congolese scientist on attachment to the University of Louvain.

In CIRAD the micro-satellite approach will commence in 2^{nd} year of the project.

Results of the molecular and VCG analyses will ultimately be compared to those for other approaches, e.g. specificity and aggressiveness of isolate to different coffee types, to determine if any correlation exists and, where possible, to identify specific markers for particular traits.

Task 6 (CABI): Synthesis of the results of all above tasks in order to propose explanations on the evolution of the fungus

Key findings from the various research activities being undertaken by the work package partners is being collated by CABI. Collation and analysis of data and interpretation of findings is being facilitated by establishment of the electronic database to which data is as and when available. As the work package research has only recently been initiated it much too early to try to draw any conclusions from the activities undertaken to date. However, this report summarises the activities undertaken to date and provides an overview of the progress being made.

Achievments

Task 1 & 2

- Collection of strains of *Fusarium xylarioides* from representative area of robusta culture in Uganda.
- Cloning by Single sporing of around 100 isolates in progress (CORI, CIRAD, UCL).
- Reception and providing of isolates between partners with appropriate quarantine restriction.
- Development of an electronic database

Task 3

- (See task 1 WP2)

Task 4

- No activities in Year 1

Task 5

- Molecular variability using variable number tandem repeat (VNTR) primers and digestion of the rDNA IGS give rise to almost identical fingerprint profiles for all strains (CABI)
- Five ISSR PCR primers, revealed no varition between the strains but each of the remaining primers demonstrated some level of intraspecific variability (CABI)
- Vegetative compatibility group (VCG) identification, in progress, mutants typed (CABI)

Still to be done

Task 1 & 2

- Collect of RDC geographical isolates to be done.
- Completion of the collection of isolates on identified genotypes in Kituza (Uganda).

Task 3

- Confirm the first results on another isolates

Task 4

- Activities relating to task 4 will begin in UCL in 2003

Task 5

- Activities relating to task 5 will commence in CIRAD in 2003

WP2 - Host/Pathogen interaction

Task 1 (CIRAD-UCL-CORI-UNIKIN) Identification of isolates of Fusarium representing a wide range of host susceptibility/resistance by screening tests on seedlings both in Africa and in Europe using available germplasm.

The development of a standardised technique of inoculation is a priority to evaluate the intensity of symptoms with a high reliability between time repetition and field resistance.

In CIRAD, an inoculation technique by injecting with a syringe a calibrated suspension of conidia in a stem of a entire plant in controled conditions (photoperiod 12h/12:, 25° or 28°C, artificial light) , with a statistical design based on the Generalised Linear Model.

Due to the low quantities of seedlings available not enough replications could be made.
However we were able to achieve 2 replications in the time for 9 population-varieties with 2 isolates. Despite a variation in the results no noticeable change in the range is observed except for population 1178 that looked almost resistant in one case (16% dead) and very susceptible in the other case (92% dead). For these 2 series the same variation, in the same proportion was observed for isolate RDC002. This variation may be due to the physiological stage of the seedling (age).
The systematic use of a water control in all trials is a proof that the technique used does not harm the plants and has no influence on the expression of symptoms.

In any case this result shows the influence of environmental condition, especially temperature, on the experiment and on the expression of symptoms. This implies to introduce a control in every trial, so as to ascertain the validity of the trial over time

The standard inoculation technique retained at this stage is syringe inoculation of a calibrated 1 x 10-6 conidia/ml suspension in 8 to 10 months old plants, that are placed at 25°C with a 12/12 hours photoperiod for incubation. This technique must be validated with all the partners.

This technic allowed to evaluated aggressivness of a set of isolates. A variation like a continuum in aggressivness of the different isolates tested is observed. These isolates will be firstly analysed with molecular techniques and VCGs.

CORI develop two techniques of inoculation on entire plants 8 to 10 months old, by root dipping in a calibrated suspension of conidia and by injection in the stem.

UNIKIN must develop an inoculation technique on young seedlings with two pairs of leaves.

These first results permitted to identified tester isolate for routine test in CIRAD (CAB003) and in progress in CORI'.

Task 2 (CORI-UNIKIN) Conduct similar field inoculations in Africa to validate inoculation method

No field inoculation was developed, but planting of healthy plants in infected soils is considered.

Task 3 (CIRAD) From the outcomes of T1 and T2 the compatible-incompatible interactions will be observed trough a histological study of all stages from penetration to host necrosis and pathogen sporulation

Detailed information on the infection process, from penetration until spreading of the pathogen in host tissue are very important, especially at the early stages, because one objective was to develop a bio test on leaves. The inoculation techniques, dipping the root or injection by syringe are destructive. It's not possible to repeat the inoculation and the plant is destroyed.
The observation of the leaf area by Scan Electron Microscopy (SEM) shows that *Fusarium xylarioides* does not develop any appressorium-like penetration structure on the leaf area, and that stomata are not use as ways of penetration of the fungus.

However if the tissues are wounded mycelium penetrates and develops by colonising the cells of the spongy mesophyll. No external symptoms indicate the fungus' presence inside the tissues.
A wound is thus absolutely necessary for infection to start. In these conditions a bio-test on leaves seems difficult to developpe.

In the case of 18 months old plants mycelium invades xylem and phloem, and develops a dense hyphal colonisation that are thought to block water and sap transportation. The cells as well are invaded and deteriorated. Inside the plant, the fungus seems to develop inside the vessels, but also through the intercellular space.
However, cuts at various internode levels of plants that exhibit the early symptoms of the disease do not allow localising the fungus. Either the cuts were made in zones where the fungus was absent despite early symptoms, or mycelium had not reached that level of the plant.
This fungus may act by blocking the water and sieve fluxes in xylem and phloem fibers. One point to strengthen this hypothesis is that, in many cases, suckers develop below the inoculation point. However, in most cases these suckers become sick rapidly, and do not survive.

In one plant, 9 months old entire plant, with apparent symptoms, whatever the internode level, no mycelium was found inside the tissue, but the cells and the structure was partially deteriorated. Remote reaction of these cells to a stress induced by a mycotoxine may be envisaged.

Achievements

Task 1
- Development in progress (CIRAD-CORI) of a standardised inoculation technique
- Development of a common statistic design (CIRAD-CORI-UNIKIN) and a common symptoms scoring
- Identification of a isolate tester in CIRAD (CAB003) and in CORI
- Continuum in aggressivness of different isolates tested is observed

Task 2
- Green house inoculation in progress in Uganda (CORI)

Task 3
- First cyto histological description of a compatible interaction

Still to be done

Task 1
- Standardisation of the testing technique for all partners
- Confirm tester isolate for routine tests in CORI
- Identify a tester isolate in UNIKIN
- Develop a "Ring-Test between the different partners (CORI-UNIKIN-CIRAD-UCL)

Task 2
- Increase the number of genotypes inoculates in green house to confirm field resistance(CORI-UGANDA)

Task 3
- Cyto-histological description of an incompatible interaction

Task 4
- Too early to be envisaged

WP3 - Breeding for resistance

WP3 Task 1 Identify sources of resistance through field assessments

The search for resistance involves assessment of germplasm locally available in Uganda and DRC, and from exotic sources, mainly other African countries with history of having controlled CWD using resistant varieties.

Uganda

On station (CORI Kituza and KARI Kawanda): *C. canephora*, *C. arabica*, Arabusta, and other coffee species are being assessed. A variety trial field at Kituza exposed to CWD has exhibited differences in variety responses to the diseases. Some of the lines in this trial have remained unaffected by the disease and they could be resistant to CWD and therefore are ideal for inclusion in the screen house assessment.

Table 3.1: Clone Trial, Kituza 1997. %CWD incidence, May 2002 (4 blocks of 6 trees per clone).

Clone	Healthy %	Symptoms %	Dead %	Total Sick
J/1/1	100	0	0	0
Q/3/4	95,8	4,2	0	4,2
1/S/3	*87,5*		*12,5*	*12,5*
1S/2	*87,5*	*4,2*	*8,3*	*12,5*
C/6/1	83,3		16,7	16,7
L/2/7	66,7	0	33,3	33,3
223/32	*58,3*	*16,7*	*25*	*41,7*
G/3/7	58,3	4,2	37,5	41,7
B/1/1	54,2	9,3	37,5	46,8
R/1/4	54,2	0	47,5	47,5
B/2/1	50.0	4,2	45,8	50.0
P/3/6	12,5	4,2	53,3	57,5
C/1/7	41,7		58,3	58,3
257/53	*29,3*	*8,3*	*62,5*	*70,8*
P/5/1	29,2		70,8	70,8
Q/1/1/	25.0		75.0	75.0
Q/6/1	25.0	4,2	70,8	75.0
B/6/2	25.0	0	75.0	75.0
E/3/2	20,8	8,3	70,8	79,1
H/4/1	12,5	4,2	83,3	87,5

Italics: progenitors of hybrids

Off station: Local germplasm also include the coffee trees surviving in wilt 'hot spots'.
A baseline survey was conducted between March and May 2002 in Uganda. It was established that in the districts of Mukono, Bundibugyo, Rukungiri, Kayunga, Kibale, Hoima, Kiboga, Mubende, Luwero, Wakiso and Mpigi, there are coffee farms which, are nearly 100% devastated. However a few scattered surviving genotypes were found. These farms are therefore potential sources of additional materials for screening.

DR Congo

Assessments on the reaction of various planting materials to the disease will be made at Yangambi, a Station of INERA (Institut National pour l'Etude et la recherche Agronomique). This station lays along river Congo, at 470 m a.s.l. The climate is continental equatorial without clear dry season. Rainfall can reach 2,000 mm, average minimum temperature is 19.5 °C, and average maximum temperature is 30°C, humidity is close to saturation.
CWD was observed for the first time in 1939 at Yangambi on plant material originating from Bangui (Central Africa). Later on in 1950, attacks reaching 30-40% dead trees were mentioned in some of the coffee fields. Assessments have been made since 1977; some are reported in Table 3.1.
Results in Table 3.2 indicate that clones L251, L215, and L147 are the most susceptible, followed by clone SA158. Only clone L93 looks less susceptible. Petit Kouillou is susceptible but is less attacked than other clones. In the hybrids only L93 x S1 is affected, at a very low level. Unlike their parents in the clone trial, the progenies of Clones L147 and L251 have a very low level of attacks. Other hybrids established in this trial do

not have enough plants to make any conclusion at this stage. Results that are more detailed will be reported after we visit the station. In addition, during the visit to the station and to other sites classes of reaction to the disease will be defined.

Table 3.2: CWD infection levels in trial fields, Yangambi, January 2000
Percentage of plants dead, deaths being attributed to CWD

Field	No.	Entry	Plants	% dead
"Parc à Bois"	1	Clone L251	33	55%
	2	Clone L215	30	50%
	3	Clone SA158	21	35%
	4	Clone L147	35	58%
	5	Clone L 93	10	17%
"Petit Kouillou"	1	Population PK	8	13%
	2	Progeny S21 (*)	10	17%
	3	Progeny LAF 159 (*)	17	28%
	4	Progeny L 147 (*)	11	18%
	5	Progeny L 251 (*)	11	18%
"Robusta x Petit Kouillou" hybrids	1	Progeny L 251 (*)	44	16%
	2	Progeny L 147 (*)	44	2%
	3	L 93 x S1	32	9%
	4	L 93 x S23	22	0%
	5	SA 158 x S9	12	0%
	6	L36 x S1	11	0%

(*) Free pollinated progeny
"Parc à Bois", 1964: rows of 10 trees, 6 Blocks
PK = Local adaptation, Petit Kouillou, 1989, rows of 10 trees, 6 Blocks
Hybrids: No replication

WP3 Task 2 Collect seeds from genotypes representative of the available germplasm

Uganda

Harvesting and rooting of cuttings at CORI coffee nursery has started. From 15/5/02 to 29/8/02, 163 genotypes have been rooted; some seeds have already been collected. The Expected date due for inoculation is between November 2002 and February 2003. Seeds of most of these genotypes will be harvested during November 2002-January 2003 harvest season.

DR Congo

For the Robusta variety, 7 clones are currently released to the farmers, namely L36, L48, L93, L147, L215, L251, and SA158. This material is available and well identified at Yangambi Research Station, which is just becoming reachable. They will be regularly harvested for dispatch to the partners. For the Bas Congo region the list of recommended clones is different (Petit Kouillou variety), namely: LAF93, LAF159, S9, S19, S23. At Kiyaka Research Station, two Robusta blocks are established from materials selected at Yangambi. High yielding individual trees have been mass selected, and are being used as a source of seeds for screening. Seeds will be regularly collected at Yangambi and Kiyaka, and at Luki, for Robusta and Kouillou respectively.

WP3 Task 3: Conduct screening tests both in Africa and in Europe using isolates with a wide range of aggressiveness.

In Uganda, seedlings and cuttings of all genotypes being assessed will be inoculated using protocols developed in partnership with pathologist. The inoculated plants will be scored for response to CWD infection. Wilt resistant genotypes will be selected and planted in multiplication plots at CORI.

In DR Congo, no results available yet

The first tests at Cirad were started in order to validate the inoculation technique at a wide scale, and to look for resistance factors within a wide representation of the 5 identified genetic groups within the *Coffea canephora* species. Five trials were undertaken on 8 to 10 months old plants.
The protocol was similar to the one described in the Chapter "Elaboration of a standard inoculation technique", and analyses were made using the General Linear Model.

The results as a whole indicate a rather good repeatability. The lack of seedlings did not allow a sufficient number of replications at different dates. Thus no statistical analysis can be done. The systematic use of a water control in all trials showed that the technique itself had no influence on the expression of symptoms. Therefore, this water control will not be used any further.

This first series of tests used *Coffea canephora* populations that can be considered representatives of the five genetic groups identified within the species. At a first glance, these populations express various levels of resistance with isolate CAB003.
Populations 1160, 1173, 1219, and 1230 look resistant to *F. xylarioides*. (To be confirmed)
For some populations (Table 3.3) the response is clear, namely 1213, 1153, 1191, 1236 (susceptible) or 1219 and 1160 (resistant). For others the rate is intermediate, around 50%. Limits between these intermediate levels of resistance cannot be defined yet. These results can be considered a trend, and will have to be confirmed. However, it can be noted that some genotypes have already been identified as possible sources of resistance.

WP3 Task 4: Prepare and establish multilocational field trials with tolerant varieties

Cuttings will be harvested from the CWD resistant/tolerant lines in the multiplication gardens and rooted in a nursery at CORI. The rooted cuttings will be planted in on-farm and on-station field trials in CWD 'hot spots' in different coffee growing locations. The on-station trials will be at CORI. The lines planted in multi-location trials will be assessed for response to CWD under field conditions under different farmers' management and different environments. They will also be assessed for yield, quality and resistance to other major coffee diseases such as red blister and leaf rust.

WP3 Task 5: Study the inheritance of the resistance

WP3 T5.1 Assessment of segregation in F2 seeds derived from Resistant x Susceptible cross (Robusta): **Uganda**

Note: Some of the clones in the clone trial are hybrids themselves: both parents and the clone itself will be sampled to assess F2 segregation (nursery, and field)

Based on the clones data (Table 3.1) and on the list of hybrids established (Table 3.4) it is proposed to test the resistance of F2 progenies from three hybrids, namely:
1s/2 x 257/53 (MR x S)
1s/2 x 223/32 (MR x MS)
1s/3 x 223/32) (MR x MS)
1s/2 x 1s/3 (MR x MR).

Table 3.3: Mortalities per progeny, per isolate, and per trial. Trials 8 to 12 Cirad

	Grey= Replicated		Isolate	
Progeny	**Mother parent: origin**	**Trial**	**CAB003**	**RDC002**
1143	Clone 119	9	25	16
1143	Clone 119	8	83	42
1143	Clone 119	10	25	33
1143	Clone 119	12	50	
1151	Clone 529	11	66	66
1153	C. liberica liberica	12	86	
1157	*C. canephora* Nana	11	66	58
1158	*C. canephora* Nana	8	75	42
1158	*C. canephora* Nana	11	83	50
1160	*C. canephora* Nana	12	11	
1162	Congolais	8	67	50
1162	Congolais	11	100	92
1164	Congolais	10	50	25
1167	Kouilou	8	75	42
1167	Kouilou	11	100	67
1169	Kouilou	9	33	0
1172	Guinean	8	0	0
1172	Guinean	11	42	42
1173	Guinean	8	0	16
1173	Guinean	11	8	0
1177	Clone 197	8	58	50
1177	Clone 197	11	84	100
1178	Clone 197	10	16	16
1178	Clone 197	11	92	92
1181	Clone 200	9	33	33
1183	Clone 202	10	25	50
1184	Clone 202	9	33	16
1186	Clone 400	9	75	42
1186	Clone 400	11	84	84
1189	Clone 503	9	24	16
1191	Clone 503	12	78	
1197	Group C	10	42	16
1197	Group C	12	35	
1208	Group: SG2	9	50	42
1213	Group: SG2	12	92	
1215	Guinean	12	65	
1219	Guinean	12	14	
1227	Group B	12	64	
1235	Group C	12	54	
1236	Group C	12	71	

Table 3.4: Hybrid trial, Kituza 1997. List of hybrid progenies (2 replications of 6 trees in rows per progeny/clone)

Entry No.	Mother Clone	Father Clone	Entry No.	Mother Clone	Father Clone
1	1s/2	Clone	24	245/62	1s/2
2	1s/2	1s/3	25	245/62	1s/3
3	1s/2	223/32	26	245/62	1s/6
4	1s/2	257s/53	27	245/62	223/32
5	1s/3	Clone	28	245/62	258s/24(0)
6	1s/3	223/32	29	257s/53	Clone
7	1s/3	259s/56	30	258s/24(0)	Clone
8	1s/3	J1/14	31	258s/24(0)	1s/6
9	1s/6	Clone	32	258s/24(0)	223/32
10	1s/6	1s/3	33	258s/24(0)	245/62
11	1s/6	223/32	34	258s/24(0)	259s/56
12	1s/6	257s/53	35	259s/56	Clone
13	1s/6	J1/14	36	259s/56	1s/2
14	1s6	259s/56	37	259s/56	245/62
15	223/32	Clone	38	259s/56	257s/53
16	223/32	1s/6	39	259s/56	258s/24(0)
17	223/32	258s/24(0)	40	J1/14	Clone
18	236/26	Clone	41	J1/14	1s/2
19	236/26	1s/2	42	J1/14	1s/3
20	236/26	245/62	43	J1/14	245/62
21	236/26	259s/56	44	J1/14	258s/24(0)
22	236/26	J1/14	45	J1/14	259s/56
23	245/62	Clone	46	J1/14	259s/56

WP4 - The disease: epidemiology

Task 1 (CORI, UNIKIN, CIRAD): Identify sites where the epidemic can be surveyed over 4 years. Characterise them regarding climate, soil, variety, type of farm and cultural practices.

In DRC, the coffee regions can be split into 3 main sectors:
- Southern: Bandudu, Bas Congo, Western and Eastern Kasai provinces
- Northeast: North Kivu provinces (Beni and Butembo)
- Northern: Eastern Equator provinces (Kisangani and Yangambi)

Two types of plantations can be found.
- Village smallholdings, mainly gardens close to the houses or plants scattered. The average area is .8 to 1.3 ha.
- Large agro-industrial units that belong to local or foreign companies or individuals. The areas are above 50ha.

The part of smallholders in the country's production is about 70%.
In Bas-Congo the average area per farm is 1 ha except for the Kakongo community where small farms can easily reach 4 ha. In Mweka territory (Western Kasai) most farmers have access to grouped coffee garden that average 2-3 ha each. In Bandundu, the areas are small. Many industrial farms were being abandoned mainly due to economic difficulties and to the lack of manpower, or was converted to other crops.

In Bas-Congo the variety Petit Kouillou is widely used. It is made of a mixture of 5 selected clones, namely L93, L159, S9, S19, and S23. INERA Research Station at Luki provided this material to the farmers.
In Bandundu and Kasai, the variety grown is Robusta, selected and distributed by Bena Longo and Mukumari (Sankuru) INERA Stations. We were told that the initial material came from Yangambi, and comprised the standard clone material i.e. clones L36, L48, L93, L147, L215, L251, and SA158.
Kiyaka Research Station has 2 blocks (3 ha) established in 1954 and 1963. These Blocks are currently maintained, and will be used as a source for plant materials for our studies.
No case of the disease could be found in any of the site visited. The mortality rate is very low (less than 10%). Cases reported by farmers or by other agriculturists are obviously wilts in connection with root rots.

The epidemiological surveys in Northern and North Eastern DRC are planned for December 2002.

In Uganda, disease surveys were made as part of other European Projects. The data have not been available up to now.

Task 2 (CORI, UNIKIN): Description of the spatio temporal spread of the disease. Devise and operate the same method for the survey of the epidemic. Make sure the data collected from various sites can be compiled and analysed the same way.

In DRC Coffee farms representative of the region were sampled as follows:

a) 12 locations per site, representing one or more groups of villages
b) In each location the assessment is done for at least 5 small farms and 5 large plantations for each group of villages.
c) In each farm at least 30 trees were surveys for small farms, and more than 30 for bigger farms depending on their size.

In Uganda, four sites in small holders farms, for epidemiological observations have been selected in Mayuge and Iganga districts (Eastern Uganda) where disease pressure is low. For comparison, four sites are selected in Masakai and Rakai districts. Disease recording is planned every 4 weeks.

Task 3 (CORI,UNIKIN): Define the conditions conducive to the appearence of the sexual phase and its importance in the spread of the disease (assessment of ascospore production in the field)

Activities relating will commence in Year 2

Task 4 (CORI, UNIKIN): Evaluate in the field the ways and duration of survival of the pathogen and where it takes place (various tissues, soil).

WP3 Task 5.2 Hybridisation: Resistant x Susceptible (Robusta)

The hybridisation scheme that was undertaken in 2002 aimed at crossing clones known for their various levels of resistance. It was proposed to make a limited number of crosses, but to ensure that each hybridisation will produce enough seeds in 2003 for:

- nursery tests of progenies at CORI and at CIRAD using a range of reference isolates
- field establishment of the progenies in zones affected by CWD.

Table 5: Crosses made at Kituza. Achievements, November 2002 (flowers/fruits counted on pollinated branches)

Mother\Father	1s/2 MR	1s/3 MR	1s/6	2/7/2	202/63/1	223/32	254/80/2	257s/53	258s/24	E/3/2	Farmer rukungiri/1 R	J/1/1 Resistant	JB5109_4/5/1	P/3/6	Q/3/4
1s/2	X					130	68	42							
1s/3		X													
1s/6			X												
2/7/2	22			X		14									
202/63/1	7				X	66	3				9	121		63	103
223/32						X									
254/80/2	7	50	25		53	**208**	X	43	49		73	6	52		
257s/53								X							
258s/24									X						
E3/2										X					
Farmer Rukungiri/1	10	17	8		43	21	64	13			X	24	7		10
J/1/1 (R)	26					28		23			14	X			
JB5109_4/5/1	23					23	51		19		25		X		
P3/6														X	
Q/3/4	58					**154**		**129**		**144**				**251**	X

WP3 T5.3 Assessment of inheritance of arabica resistance

Cross infections between arabica strains (Ethiopia) and canephora and vice versa will normally be made at Cirad by Girma Adugna (EARO) in 2003 (DFID Project).
In the meantime, the existence of Arabusta F1 and F2 (backcrosses) hybrids in the field (CORI) offers a possibility to study segregation of the resistance to the Robusta strain found in Arabica.

WP3 Task 6: Propose a breeding strategy towards durable resistance
Based on studies on the screening responses and heritability, a strategy for breeding durably resistant varieties will be proposed.

WP3: Breeding for resistance: milestones

Achievements

- Uganda: identification of various levels of field resistance. DRC: Evaluation of field resistance of some clones in various geographical areas
- Signature of a MTA (Material Transfer Agreement) between CORI and partners via CIRAD
- Uganda: hybridisations undertaken to study inheritance of resistance
- Dispatch of seeds for screening

Still to be done

- Identify resistant materials (RDC)
- Confirm field and tests results, presence/absence of interaction
- Test F2 segregation of resistance (CORI, CIRAD)
- Complete crosses programme (CORI)

Activities relating will commence in Year 2

Task 5 (UNIKIN): Elaboration of a simplified model of the epidemic and proposition of adapted recommendations to the prevailling conditions in this area

Activities relating will commence in Year 3

Achievements

Task 1
- In DRC, no case of the disease could be found in any of the site visited in Bas Congo, Bandundu and Kasai Provinces

Task 2
- In Uganda, identification of sites in various districts
- In DRC activities relating will commence in Year 2

Task 3 & 4
- Activities relating will commence in Year 2

Still to be done

Task 1
- Epidemiological surveys in Northern and North Eastern DRC (UNIKIN)
- Dispatch to partners information collected by other EU funded projects

Task 2
- Devise and operate the same method for the survey of the epidemic (UNIKIN-CORI)

Task 3
- Set up the protocol proposed by UCL

Task 4
- Commence this task during year 2

Publications

COWIDI-Project. CIRAD-CORI-UNIKIN-CABI-UCL. Work-shop report "Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. Kampala (Uganda) 4 to 7/03/2002.

Meetings

BIEYSSE Daniel. The Coffee Wilt in Africa. Journées de Septembre. Montpellier (France). 28/08 to 6/09/2003

Outline Plan Year 2

Tasks		Partners	
1-1	Survey and collection of isolates	CORI	Collection of isolates on identified clones
		UNIKIN	Collection of geographical isolates
1-2	Identification, storage and exchanges of isolates	CABI	Continue the task
1-3	Evaluation of the agressivness	UNIKIN-CIRAD	Continue the task
1-4	Description of the fungus cycle	UCL-UNIKIN-CORI	Start the task
1-5	Evaluation of the genetic diversity by RAPD and microsatellites	CABI	Continue the task
		CIRAD	Start the task
1-6	Synthesis of the results	CABI	
2-1	Identification of isolates representative of genetic diversity and aggressivness	CIRAD	Continue the task
		CORI	Confirm the tester isolate
		UNIKIN	Identifie tester isolate
2-2	Field inoculations in Africa	CORI	Planting healt coffee plant in infected soils
2-3	Cyto-histological study, types of reaction	CIRAD	Continue the task
2-4	Hypothesis on the nature of the resistance	CIRAD-CORI	Partial conclusions
3-1	Identification sources of resistance in the field	CORI	Continue the task
		UNIKIN	Start the task
3-2	Collect and dispach seeds and cuttings	CORI	Continue the task
		UNIKIN	Start the task
3-3	Screening tests on seedlings and cuttings in Africa and Europe	CORI-UNIKIN-CIRAD	Continue the task-Development of a Ring Test
3-4	Multilocal trials with tolerant varieries	CORI-UNIKIN	Start the task
4-1	Identification of sites representatives of the epidemic	UNIKIN	Start the task
4-2	Description of the spatio temporal diffusion of the disease in the plantations	UNIKIN	Start the task-Development of common evaluation
		CORI	Continue the task
4-3	Definition of the conditions conducive to the sexual phase	CORI-UCL	Start the task
4-4	Evaluation of the duration of survival form of the pathogen	CORI-UCL	Start the task

MANAGEMENT ANNUAL REPORT

Organisation of the collaboration

This Project is the first opportunity for scientists from 5 countries to collaborate in the study of a major problem of Robusta coffee in Central and East Africa. Previously, each partner had been trying to develop limited and not concerted actions.

The theme is relatively new as Coffee Wilt is a re-emerging disease.

During this first year the partners have been able to establish the basis for coming work, they have been learning to know each other, and to collaborate. This was facilitated by the Workshop in Kampala 3-7 March 2002.

An essential step was reached thanks to the elaboration of rules, namely regarding the transfer of living materials to be used by the partners within the duration of the Project. The exchanges were particularly formalised with CORI by the signature of a Material Transfer Agreement (MTA)

Besides, the partners exchanged information regularly; this should lead sooner or later to standardised working procedures, taking into account local constraints.

Meetings

A launching meeting was organised in Kampala (Uganda) 3-7 March 2002. All scientists that take part in the project were present. All presentations, conclusions, and decisions on tasks sharing were presented in the first intermediary report.

No meeting is scheduled for the second year, however co-ordination will continue through exchanges of emails, and a visits by the Co-ordinator.

Exchanges

During the second year visits between the partners are scheduled as follows.

CORI-CIRAD
Mr. Pascal Musoli, Coffee Breeder, will undertake from September 2003 a PhD work with Montpellier University in France. His work will be done partly in France and partly in Uganda.

UNIKIN-UCL
Mr. Patrick Tshilenge, pathologist, will undertake DEA studies at Louvain University (UCL) from September 2003.

CIRAD-CORI
A Co-ordonation mission by the Coordinator (Daniel Bieysse, pathologist) and a Coffee Breeder (Pierre Charmetant) is planned.

CIRAD-UNIKIN
A Co-ordination mission by the Coordinator (Daniel Bieysse, pathologist) and a Coffee Breeder (Pierre Charmetant) is planned.

Problems

Some delay was experienced in the dispatch of Financial Reports; it is hope this will improve in the coming months.

CIRAD
COORDINATOR

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

First Annual Report (November 2001 to October 2002)

CIRAD-AMIS
TA40/02
Boulevard de la Lironde
34 398 Montpellier, Cedex 5,
France

WP1: Pathogen diversity

In this first year the objective has been to define the means necessary to study interactions hosts/pathogen. Indeed the prerequisite is the availability of:

- a wide range of isolates representative of the variability: this impliesto constitute and maintain a collection
- plant material representative of the known variability of C. canephora, and also of C. Arabica
- a standardised technique for inoculation and reading that gives the best possible indication on pathogenicity, susceptibility, and interaction, with reliable and repeatable results, and good correlation with field results..

Simultaneously cytologic and histologic studies were conducted by observing the first events in the colonisation of tissues, when the host recognises the pathogen and vice-versa.

Besides, the first evaluations of pathogenicity were made for a few isolates.
In connection with the availability of plant materials all the experiments to date were made using free pollinated seeds from single trees. We call this type of material "population variety".

Task 1 Collection of anamorph and teleomorph forms of the fungus on various part of the trees, possibily on alternative hosts, in affected regions. Maintenance and dispatch to European labs.

Isolates have been received from various collections (UCL, CABI, CORI, UNIKIN). Exchanges have been made with CABI and UCL.
Surveys have been made in Uganda in November 2001, February 2002, April 2002. Isolates collected in Uganda and purified in CIRAD were dispatched to CABI
The isolates are kept on PDA medium at 4°C.
The list of isolates available purified single spore is in annex 1.

Task 3. Evaluation of the variability in aggressiveness using standard inoculation test.

Isolate aggressivness

Detailled results are presented in WP2 task1.

Tab 1 General and preliminary evaluation of the aggressivness of some isolates (to be confirm)

Population	CAB003		RDC001		RDC002		OUG008		OUG022		OUG031		OUG033		OUG036		OUG057		OUG072	
	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°
1143	+++	+		+	++	+		+		+				+	++	+	-	-		
1144	+++	+		-	++	+		+		+				+	-	-	-	-		
1151	+++	+			++	+	+	+			++	-	-	-	+++	-	+++	+++	++	++

+++ high aggressivness
++ intermediate aggressivness
+ low aggressivness
- no symptoms

A preliminary evaluation of the aggressivness of isolates is indicated in table 1. These results will must be confirm.

Task 4 Description of the fungal life cycle, asexual and sexual phases

The objective is to characterise the pathogen using simple biological markers in order to have a first sight of the diversity of *F. xylarioides*. Morpho-metric aspects, growth rates on artificial medium at various temperatures, size of *conidia*, and kinetics of formation of micro- and macro-*conidia* have been observed.

4.1 Material and methods

4.1.1 The fungus

Two trials were made at two different dates. Thirteen, and ten mono-*conidia*l isolates from various origins in Uganda were used for each trial respectively.

Table 2: Origins of the 23 Fusarium sp. isolates used for the morphologic and cultural study

	Isolate	Origin	Pathogenicity
TRAIL 1	CAB003		+
	OUG007	Near Kampala	?
	OUG008	"	+
	OUG009	Hoima District	?
	OUG010	"	?
	OUG012	"	?
	OUG013	"	?
	OUG014	Kituza	?
	OUG015	"	?
	OUG016	"	?
	OUG017	"	?
	OUG018	"	?
	OUG019	"	?
TRAIL 2	CAB003		+
	OUG008	Near Kampala	+
	OUG022	Luwero District	+
	OUG031	Mukono District	+
	OUG033	Massaka District	+/-
	OUG036	Kyenjojo	+
	OUG057	Mubende	+
	OUG072	Jinja	+
	RDC001		+/-
	RDC002		+

CAB003 and OUG008 are controls for both trials.

4.1.2 Effect of temperature on growth

The strains were cultured on PDA medium in 90 mm Petri dishes, at 20, 25, and 30°C, in the dark. 3 replications per treatment were made. Growth was assessed every 2 days.

4.1.3 Morpho-metric characterisation of the *conidiae*, and cinetics of appearing.

Trial 1

Conidiae from all isolates were sampled after 10 days at 25°C, and were coloured with cotton blue. In the case of isolates OUG008, OUG017, and OUG018, *conidiae* were sampled after 72 h, 96 h 120 h, 7 days, 10 days, and 3 weeks.

Trial 2

Conidiae were sampled on 28th day at 20°C for all 10 isolates, whereas for isolates CAN003, OUG008, OUG033, and OUG072, they were sampled from the 25 and 28°C treatments.

For both trials, ANOVA and Newman & Keuls tests (p=0.05) were performed using Statistica software.

4.2 Results

Trial 1

Effect of temperature on mycelium growth, and on cinetics of *conidiae* apparition.

Table 3 shows the growth rates of each strain and temperature. Growth rates are similar at 20 and 25°C; a slight inhibition is noticeable at 30°C (Figure **4**).

The classes of growth rates depend on the maximum average growth after 7 days at 25°C. For all temperatures, data analysis shows 3 classes of isolates: some with slow growth, some with fast growth, and an intermediary class (Table 9). The evolution of means over time is given in Figure **4**.

Table 3: Mean diameter (mm) of colonies after 7 days at 3 temperatures

Temp. Time Isolate	20°C			25°C			30°C			Class of growth rate
	48h	96h	7 days	48h	96h	7 days	48h	96h	7 days	
OUG015	7,3	13,5	33,6	8,3	17,2	29,7	07,1	19,8	26,5	L
OUG014	8,6	15,2	34,4	8,6	17,7	30,5	07,3	20,2	25,8	L
CAB003	7,9	17,1	30,3	8,5	18,7	31,1	6,6	12,2	21,3	L
OUG008	7,2	20,75	30,2	9,6	19,8	31,8	6	13,2	23,2	L
OUG019	8,8	13,8	34	8,5	17,5	34,8	7,2	21,8	28,2	L
OUG009	7,7	15,7	28,7	9	19,8	34,9	8,4	16,1	26,9	L
OUG007	6,5	16,6	36	9	20,9	38,4	7,3	14,4	28	L
OUG016	13,2	28,4	53,1	16,2	34,3	60,41	15	48,5	59,2	I
OUG012	12,6	24,8	43,3	15,9	34,9	64,2	15,4	32,9	57,3	I
OUG010	21,7	37,4	70,6	2,,4	43,2	65,6	18,8	38,2	64,3	I
OUG013	17,7	49,1	85	17,3	51,6	85	18,1	5	85	R
OUG018	21,8	42,5	76,8	26,7	53,8	85	22,2	70,5	80,3	R
OUG017	22,3	49,2	85	25,9	55,2	85	22	67,8	78,8	R

Classes: R = fast growth, I = Intermediate growth, L = Slow growth

Characterisation of strains by the type of *conidiae*

Mean sizes and standard deviation for each strain are presented in table 4. The Newman & Keuls test (p=0.05) defines 5 groups of non-significantly different means. The limit between micro- and macro-*conidiae* can clearly be set at 20 x 10^{-6} m.
The distribution of the data is presented in Figure 5.

Table 4: Characterisation of strains by the mean size of their *conidiae* (10 days)

Isolate	Mean size (µm)	SD	Group(*)	Type of *conidiae*
OUG009	7,3	1,9	a	Micro
OUG015	8,1	9,1	a	Micro
OUG008	8,4	2,6	a	Micro
OUG019	8,4	4,2	a	Micro
OUG007	8,5	3,4	a	Micro
OUG014	8,6	3,2	a	Micro
CAB003	8,7	2,1	a	Micro
OUG016	12,1	2,5	b	Micro
OUG012	13,2	3,1	b	Micro
OUG018	26,4	5,7	c	Macro
OUG010	32,4	7,5	d	Macro
OUG013	34,4	6,6	d	Macro
OUG017	36,7	5,0	e	Macro

(*) means followed by the same letter are not significantly different (Newman & Keuls, p=0.05)

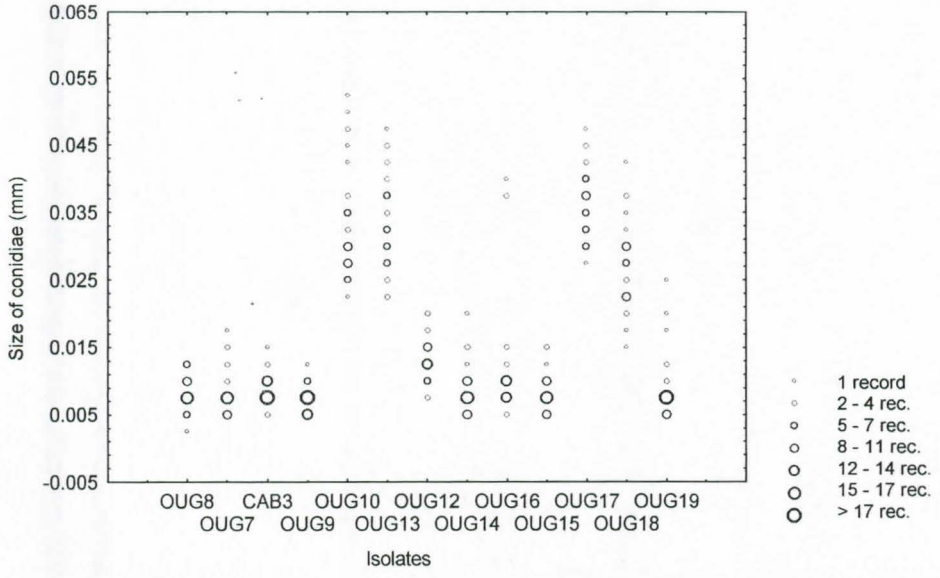


Figure 2: Distribution of classes of *conidiae* size for 13 *Fusarium sp.* Isolates after 10 days culturing

Cinetics of apparition of *conidiae*

One isolate representative of each group was chosen for this study, i.e. OUG008 for micro*conidiae*, OUG00017 for macro*conidiae*, and OUG0018 for intermediate size.

Table 5. Cinetic of apparition of conidae type of Isolate OUG008 (Mean size and homogeneous groups)

Time (days)	Mean size µm	% micro *conidiae*	% macro*conidiae*	Group	Dominant type of *conidiae*
3	10.3	100.0	0	a	Micro
4	12.4	92.5	7.5	a	Micro
5	12.8	100.0	0	a	Micro
6	13.0	100.0	0	a	Micro
7	11.4	95.0	5.0	a	Micro
11	12.1	97.5	2.5	a	Micro
21	23.1	50.0	50.0	b	Macro

The size of the *conidiae* remains stable over time, up to 21 days when one can observe a split into 2 populations. The mean size after 21 days is significantly different from the previous days (Newman & Keuls, p = 0.05). This suggest that macro*conidiae* appear after micro*conidiae*.

Table 6. Cinetic of apparition of conidae type of Isolate OUG017 (Mean size and homogeneous groups)

Time (days)	Mean size µm	% micro *conidiae*	% macro*conidiae*	Group	Dominant type of *conidiae*
3	42,8	0	100	a	Macro
4	25,2	7,5	92,5	a	Macro
5	42,5	0	100	a	Macro
6	46,3	0	100	a	Macro
7	40,9	2,5	97,5	a	Macro
11	51,6	0	100	a	Macro
21	31,7	10	90	b	Macro

The size was constant over time. Only macro-*conidiae* appeared. No significant difference could be found whatever the date. The proportion of micro-*conidiae* was always lower than 10%.

Table 7. Cinetic of apparition of conidae type of Isolate OUG008 (Mean size and homogeneous groups)

Time (days)	Mean size µm	% micro *conidiae*	% macro*conidiae*	Group	Dominant type of *conidiae*
3	32	0	100	a	Macro
4	38	0	100	a	Macro
5	37	0	100	a	Macro
6	38	0	100	ab	Macro
7	32	2,5	97,5	ab	Macro
11	34	7,5	92,5	ab	Macro
21	33	0	100	ab	Macro

The average size was constant over time. Isolate OUG018 produced a large majority of macro-*conidiae*.

Summary of results, Trial 1

Table 8: Summary of results, Trial 1, at 25°C

Isolate	Type of growth	Type of *conidiae*	Colour of mycelium
OUG009	L	Micro	Rosé
OUG015	L	Micro	Rosé
OUG008	L	Micro	Rosé
OUG019	L	Micro	Rosé
OUG007	L	Micro	Rosé
OUG014	L	Micro	Rosé
CAB003	L	Micro	Rosé
OUG016	I	Micro	White
OUG012	I	Micro	Rosé
OUG018	I	Macro	White
OUG010	R	Macro	Rosé
OUG013	R	Macro	Rosé
OUG017	R	Macro	White

L = slow growth, I = Intermediate growth, R = Fast growth

This preliminary study allowed us to identify two groups in this population of *Fusarium sp.* Isolates from Uganda, namely:

- one group that produces a majority of micro-*conidiae*, and grows slowly
- another group that mainly produces macro-*conidiae*, and grows fast.

TRIAL 2

Effect of temperature on mycelium growth

Tables 9: Growth rate of mycelium of 10 *Fusarium sp.* Isolates under 3 temperatures.

	Isolates	Mean growth rate(mm)	a	b	c	d	e
Temperature 20°C	OUG 72	50,00	x				
	OUG 31	67,00		x			
	CAB 3	72,00		x	x		
	OUG 8	72,17		x	x		
	RDC 1	74,33		x	x		
	OUG 22	78,33		x	x	x	
	RDC 2	81,75			x	x	x
	OUG 36	86,67				x	x
	OUG 57	90,00				x	x
	OUG 33	90					x
Temperature 25°C	OUG 57	67,83	x				
	OUG 22	69,83	x				
	OUG 31	75,67	x	x			
	OUG 72	75,83	x	x			
	OUG 8	77,17	x	x			
	RDC 2	77,17	x	x			
	CAB 3	80,67	x	x	x		
	RDC 1	83,83		x	x		
	OUG 36	84,83		x	x		
	OUG 33	90,00			x		
Temperature 30°C	OUG 8	48,0	x				
	OUG 57	52,2	x				
	OUG 22	56,8	x	x			
	OUG 72	57,0	x	x			
	RDC 2	58,2	x	x			
	OUG 31	65,5		x			
	CAB 3	65,5		x			
	RDC 1	67,8		x			
	OUG 36	82,0			x		
	OUG 33	90,0			x		

At 20°C isolate OUG072 is significantly slower than the other while OUG033 is the fastest. Also, OUG057 grows significantly faster than RDC002. OUG057, also the fastest at 20°C, is amongst the slowest at 25 and 28°C.
Analysis for 25°C splits the growth means into 3 homogeneous groups, however no isolates appears significantly different from the others. OUG033 is as fast as at 20°C while OUG057 is much faster.
At 28°C OUG036 and OUG033 are significantly faster than all others. OUG008 and OUG057 are significantly slower than OUG031, CAB001, and RDC001.

Characterisation of strains by the type of *conidiae*

Table 10 compares means and Standard Deviations observed for 9 isolates at 20°C, and for 4 of them at 25 and 28°C. RDC001 did not produce *conidiae* at all. ANOVA was performed for data at 20°C only.

Table 10: Mean size of *conidiae* (in µm.)and Standard Deviation for 9 isolates)

Temperature	20°C			25°C		28°C	
Isolate	Mean(µm)	SD	Group (*)	Mean(µm)	SD	Mean(µm)	SD
OUG072	5.73	1.40	a	5.85	1.17	6.20	1.51
CAB003	6.30	1.40	a b	8.30	1.79	6.48	1.57
OUG022	6.60	1.30	b	N/A	N/A	N/A	N/A
OUG036	6.77	1.33	b	N/A	N/A	N/A	N/A
OUG008	6.82	1.57	b	5.60	1.17	6.05	1.15
OUG057	6.90	1.85	b	N/A	N/A	N/A	N/A
RDC002	7.00	1.28	b	N/A	N/A	N/A	N/A
OUG031	7.10	1.43	b	N/A	N/A	N/A	N/A
OUG033	11.00	2.24	c	10.82	2.40	9.85	2.06

(*) means followed by the same letter are not significantly different (Newman & Keuls, p=0.05)

At 20°C the smallest *conidiae* –significantly smaller than most others - are produced by OUG072, whereas OUG033 produces significantly larger *conidiae* than all others.

Table 11: Summary of results, Trial 2

	Type of growth	Size of *conidiae*
RDC001	Slow	Absence
OUG072	Intermediate	Micro
CAB003	Intermediate	Micro
OUG022	Fast	Micro
OUG036	Fast	Micro
OUG008	Fast	Micro
OUG057	Fast	Micro
RDC002	Fast	Micro
OUG031	Slow	Micro
OUG033	Fast	Macro

Unlike in Trial 1, no obvious relation can be seen between growth speed and size of *conidiae*.

Conclusions

The assessment of morphological and cultural characteristics, at various temperatures, of the available *Fusarium sp.* isolates collected on Coffee, allowed us to identified simple markers to differentiate them.

One aim of the first part of the study was to identify the optimum temperature for growth, and possibly to find a inhibiting temperature. The optimal growth is reached between 20 and 25°C. Culture made at 30°C had a relatively slow growth, especially for slow strains. However they continued growing regularly, so that 30°C cannot be considered as inhibiting.
One could see that micro-*conidiae* were always born before macro-*conidiae*. One important consequence is that cultures to be used for inoculations have to be kept for at least 3 weeks, as the respective roles of the two types are not known. Another step will be to determine which type is more pathogenic.

A relation was found between growth and size of *conidiae* (Table 5 Summary). It seems that strains with slow growth produce mainly micro-*conidiae*, and that isolates with fast growth develop mainly macro-*conidiae*.
Besides, the isolates that were found to be pathogenic generally have a slow growth, but there is no reciprocity, as OUG033 grows fast and is pathogenic.

Indeed this type of marker may help to confirm whether an isolate belongs to the species *F. xylarioides*. However it cannot be an indicator of pathogenicity. The only way to assess pathogenicity remains artificial inoculation.

CIRAD Ref	Origin Ref	District/Village	Latitude	Longitude	Collection	Brut	Single spore	Observations	Forward
OUG 001					before 08/97	+		Intro Berthouly culture remise par georgina Hakiza	
OUG 002					before 08/97	+		Intro Berthouly culture remise par Georgina Hakiza	
OUG 003					08/97	+		Intro Berry Plantation paysanne proche Kampala	
OUG 004					08/97	+		Intro Berry Plantation paysanne proche Kampala	
OUG 005					08/97	+	+	Intro Berry Plantation paysanne proche Kampala	
OUG 006					08/97	+	+	Intro Berry Plantation paysanne proche Kampala Cultures rouges	
OUG 007					08/97	+	+	Intro Berry Plantation paysanne proche Kampala	To CABI
OUG 008					08/97	+	+	Intro Berry Plantation paysanne proche Kampala	To CABI
OUG 009	1	Hoima district. Kiryangobe village			30/04/01	+	+	Petit Huguenin Croiss. Lente Plant° paysanne	To CABI
OUG 010	1'	Hoima district. Kiryangobe village			30/04/01	+	+	Petit Huguenin Croiss. Rapide Plant° paysanne	To CABI
OUG 011	2	Hoima district.Burindi village			30/04/01	+	+	Petit Huguenin Croiss. Rapide Plant° paysanne	
OUG 012	2'	Hoima district.Burindi village			30/04/01	+	+	Petit Huguenin Croiss. Lente Plant° paysanne	To CABI
OUG 013	2-1	Hoima district.Burindi village			30/04/01		+	Petit Huguenin Croiss. Rapide (issu de OUG 012 Plant° paysanne	To CABI
OUG 014	3	Kituza			30/04/01	+	+	Petit Huguenin Clonal coffee trial Croiss. Lente	To CABI
OUG 015	3'	Kituza			30/04/01	+	+	Petit Huguenin Clonal coffee trial Croiss. Lente	To CABI
OUG 016	3	Kituza			30/04/01	+	+	Petit Huguenin Clonal coffee trial colonie blanche	To CABI
OUG 017	3-1	Kituza			30/04/01	+	+	Petit Huguenin Clonal coffee trial colonie blanche	To CABI
OUG 018	4	Kituza			30/04/01	+	+	Petit Huguenin Clonal wood garden colonie blanche	To CABI
OUG 019	4'	Kituza			30/04/01	+	+	Petit Huguenin Clonal wood garden croissance lente	To CABI
OUG 020		Kituza			30/04/01	+		Petit Huguenin Clonal wood garden colonie a bords festonnés	
OUG 021	A2	Luwero District			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 022	A2	Luwero District			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 023	B	Luwero District			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 024	B	Luwero District			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 025	B	Luwero District			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 026	C	Luwero District			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 027	C	Luwero District			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 028	D	Mukono District			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 029	D	Mukono District			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 030	F2	Mukono District			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 031	F2	Mukono District			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 032	1	Massaka District Large scale plantation (600 acres			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 033	1	Massaka District Large scale plantation (600 acres			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 034	1	Massaka District Large scale plantation (600 acres			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 035	4	Kyenjojo Bwenzi vill.	0°39'08"	30°34'55"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 036	4	Kyenjojo Bwenzi vill.	0°39'08"	30°34'55"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 037	4	Kyenjojo Bwenzi vill.	0°39'08"	30°34'55"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 038	4	Kyenjojo Bwenzi vill.	0°39'08"	30°34'55"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 039	5	Kyenjojo Kasina vill.	0°37'15"	30°38'19"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 040	5	Kyenjojo Kasina vill.	0°37'15"	30°38'19"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 041	5	Kyenjojo Kasina vill.	0°37'15"	30°38'19"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 042	5	Kyenjojo Kasina vill.	0°37'15"	30°38'19"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 043	7	Kyenjojo Misanvu Vill.	0°37'15"	30°38'19"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 044	7	Kyenjojo Misanvu Vill.	0°37'15"	30°38'19"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 045	7	Kyenjojo Misanvu Vill.	0°37'15"	30°38'19"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 046	7	Kyenjojo Misanvu Vill.	0°37'15"	30°38'19"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 047	8	Kyenjojo Kabiri Vill.			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 048	8	Kyenjojo Kabiri Vill.			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 049	8	Kyenjojo Kabiri Vill.			11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	

OUG 050	8	Kyenjojo Kabiri Vill.			11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 051	10	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 052	10	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 053	11	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 054	11	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 055	11	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 056	12	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 057	12	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 058	12	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 059	12	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 060	12	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 061	13	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 062	13	Mubende Kyamwero Vill.	0°29'02"	31°37'57"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 063	16	Mubende Kyllianongo Vill.	0°36'19"	31°51'53"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 064	16	Mubende Kyllianongo Vill.	0°36'19"	31°51'53"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 065	16	Mubende Kyllianongo Vill.	0°36'19"	31°51'53"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 066	17	Mubende Nyanze Vill.	0°28'24"	31°41'52"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 067	17	Mubende Nyanze Vill.	0°28'24"	31°41'52"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 068	17	Mubende Nyanze Vill.	0°28'24"	31°41'52"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 069	17	Mubende Nyanze Vill.	0°28'24"	31°41'52"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 070	23	Jinja Nawangoma Vill.	0°31'56"	33°10'49"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 071	23	Jinja Nawangoma Vill.	0°31'56"	33°10'49"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 072	23	Jinja Nawangoma Vill.	0°31'56"	33°10'49"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 073	24	Jinja Magaluka Vill.	0°36'51"	33°11'36"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 074	24	33°10'49"	0°36'51"	33°11'36"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 075	26	Jinja Kibudaire Vill.	0°37'44"	33°08'36"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 076	26	Jinja Kibudaire Vill.	0°37'44"	33°08'36"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 077	27	Jinja Naiwansi Vill	0°38'31"	33°08'59"	11/2001	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 078	27	Jinja Naiwansi Vill	0°38'31"	33°08'59"	11/2001		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 079	AA7	Kiganda (Mubende)	0°28'42"	31°40'41"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 080	AA7	Kiganda (Mubende)	0°28'42"	31°40'41"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 081	AA22	Kiganda (Mubende)	0°29'55"	31°42'05"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 082	AA22	Kiganda (Mubende)	0°29'55"	31°42'05"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 083	AA24	Kiganda (Mubende)	0°30'07"	31°42'17"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 084	AA24	Kiganda (Mubende)	0°30'07"	31°42'17"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 085	AA33	Kiganda (Mubende)	0°28'12"	31°42'29"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 086	AA33	Kiganda (Mubende)	0°28'12"	31°42'29"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 087	AA33	Kiganda (Mubende)	0°28'12"	31°42'29"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 088	AA37	Kiganda (Mubende)	0°28'25"	31°42'45"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 089	AA37	Kiganda (Mubende)	0°28'25"	31°42'45"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 090	15	Mukono	0°29'42"	32°47'54"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 091	15	Mukono	0°29'42"	32°47'54"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 092	22	Mukono	0°30'52	32°49'30"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 093	22	Mukono	0°30'52	32°49'30"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 094	24 BIS	Mukono	0°30'52"	32°49'53"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 095	24 BIS	Mukono	0°30'52"	32°49'53"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 096	24 BIS	Mukono	0°30'52"	32°49'53"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 097	28	Mukono	0°30'38"	32°51'3"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 098	28	Mukono	0°30'38"	32°51'3"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 099	73 L	Mukono	0°31'4"	32°45'41"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	

OUG 100	73 L	Mukono	0°31'4"	32°45'41"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 101	75 M	Mukono	0°30'57"	32°45'54"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 102	75 M	Mukono	0°30'57"	32°45'54"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 103	75 M	Mukono	0°30'57"	32°45'54"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 104	77 O	Mukono	0°30'42"	32°46'22"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 105	77 O	Mukono	0°30'42"	32°46'22"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 106	78 P	Mukono	0°30'41"	32°46'23"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 107	78 P	Mukono	0°30'41"	32°46'23"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 108	80 R	Mukono	0°30'39"	32°46'24"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 109	80 R	Mukono	0°30'39"	32°46'24"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 110	80 R	Mukono	0°30'39"	32°46'24"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 111	82	Mukono	0°30'39"	32°46'15"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 112	82	Mukono	0°30'39"	32°46'15"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 113	84.2	Mukono	0°30'24"	32°46'12"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 114	84.2	Mukono	0°30'24"	32°46'12"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 115	84.2	Mukono	0°30'24"	32°46'12"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 116	86	Mukono	0°30'5"	32°46'10"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 117	86	Mukono	0°30'5"	32°46'10"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI
OUG 118	96	Mukono	0°30'54"	32°53'40"	02/2002	+		remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	
OUG 119	96	Mukono	0°30'54"	32°53'40"	02/2002		+	remote sensing Camille Lelong/FabricePinard/Georgina Hakiza	To CABI

Population	Mother parent: origin
1143	Clone 119
1151	Clone 529
1153	liberica liberica
1157	canephora Nana
1158	canephora Nana
1160	canephora Nana
1162	Congolais
1164	Congolais
1167	Kouilou
1169	Kouilou
1172	Guinean
1173	Guinean
1177	Clone 197:
1178	Clone 197:
1181	Clone 200:
1183	Clone 202:
1184	Clone 202:
1186	Clone 400:
1189	Clone 503:
1191	Clone 503:
1197	Group C
1208	Group: SG2
1213	Group: SG2
1215	Guinean
1219	Guinean
1227	B
1235	C
1236	C

Every population is issued from an individual plant excepted 1143, 1151, 1197 to 1236 (seeds in bulk from identified trees)

Preliminary Inoculation protocol

Syringe injection in the stem

Plant-material

8 to 10 months old entire plants

Inoculum concentration

Conidies issued from cultures 3-4 weeks old of monosporic isolate.
Suspension 10^6 conidies/ml
Injection above the first inter node of cotyledonnaries leaves with of a droplet more or less 30µl

Disease scoring (for routine evaluation)

Assess the initial total number of leaves of each plant
Assess the number of leaves fall down or showing wilt symptoms after 100 to 120 days post inoculation (no still definitively defined.
Give the per cent of dead plants
Give the per cent of wilt (wilted leaves/total number of leaves)

Statistic design

16 plants minimun/cultivars/isolate (this number of plants can be different if the statistic analysis is the Generalised linear model)
Introduce a control susceptible cultivar/aggressive isolate (if possible the same to evalue the effect of environemental variation on the intensity of the disease).
Statistic analysis: Generalised linear model Procedure SAS

Generalised linear model

PROC GENMOD: Introduction

SAS Institute

The GENMOD procedure fits generalized linear models, as defined by Nelder and Wedderburn. The class of generalized linear models is an extension of traditional linear models that allows the mean of a population to depend on a *linear predictor* through a nonlinear *link function* and allows the response probability distribution to be any member of an exponential family of distributions. Many widely used statistical models are generalized linear models. These include classical linear models with normal errors, logistic and probit models for binary data, and log-linear models for multinomial data. Many other useful statistical models can be formulated as generalized linear models by the selection of an appropriate link function and response probability distribution.The GENMOD procedure fits a generalized linear model to the data by maximum likelihood estimation of the parameter vector .There is, in general, no closed form solution for the maximum likelihood estimates of the parameters. The GENMOD procedure estimates the parameters of the model numerically through an iterative fitting process. The dispersion parameter is also estimated by maximum likelihood or, optionally, by the residual deviance or by Pearson's chi-square divided by the degrees of freedom. Covariances, standard errors, and *p*-values are computed for the estimated parameters based on the asymptotic normality of maximum likelihood estimators.

A number of popular link functions and probability distributions are available in the GENMOD procedure. The built-in link functions are

- identity:
- logit: probit: , where is the standard normal cumulative distribution function
- power:
- log:
- complementary log-log:
-

The available distributions and associated variance functions are

- normal:
- binomial (proportion):
- Poisson:
- gamma:
- inverse Gaussian:
- negative binomial:
- multinomial
-

The negative binomial is a distribution with an additional parameter *k* in the variance function. PROC GENMOD estimates *k* by maximum likelihood, or you can optionally set it to a constant value.
The multinomial distribution is sometimes used to model a response that can take values from a number of categories. The binomial is a special case of the multinomial with two categories.
In addition, you can easily define your own link functions or distributions through DATA step programming statements used within the procedure.

WP2: Host/Pathogen interaction

Task 1. Identification of isolates of Fusarium representing a wide range of host susceptibility/resistance by screening tests on seedlings both in Africa and in Europe using available germplasm.

1-1 Material and methods

1.1.1 Coffee plant material

Origins

C. canephora seeds were received from French Guyana, from Côte d'Ivoire, and from DRC. Unless impossible the seeds are taken from clones or from well identified individual trees. (Annex 2)

Preparation of the planting material

The seeds are germinated then raised in a glasshouse under controlled temperature (25 +- 3°C). Relative humidity is kept at 80% using a fog system. The seedlings reach the butterfly stage after 6-7 weeks. They are transplanted when they reach 2 pairs of leaves (9-12 weeks), either into individual pots or in pots of 3 or 4, depending on the inoculation technique. Due to the allogamy of the species each plant is labelled.

1.1.2 Elaboration of the inoculation technique

The objective is to make available to research a test that is reliable, reliable, standardised, and, if possible, well correlated with field results..
The usual methods are inoculation through wounding, or through dipping of bare roots into a suspension of conidiae.
Also, for practical reasons, and for standardisation, all initial tests described below were made by injecting a calibrated solution into the stem using a syringe, adapted to lab constraints.

The influence of the temperature was also studied to define the optimal condition for symptoms expression

Preparation of inoculum

Isolates are transplanted from the conservation tubes into Petri dishes on PDA medium 2 weeks before inoculation; they are kept at 25°C under a 12h/12h photoperiod. The inoculum used for inoculations is thus 2 to 6 weeks old.
In any case, unless specifically mentioned, the concentration of the solution is set at 1×10^6 conidiae/ml. The germination of the inoculum is tested after 24 hours in the dark.

Inoculation on whole plant

- 8-10 months old (at least 3 pairs of leaves)

Inoculation using a syringe to inject 20 µl of calibrated solution into the hardened part of the stem above the cotyledons. After inoculation the plants are placed in a climatic chamber at 25 or 28°C, under alternate light (12h/12h).

- 16 to 20 months old

The hole in the trunk is dug with a drill at about 10cm above the collar. 50µl solution drops are used.

1.1.3 Experimental design

In each trial, and unless otherwise specified, 16 plants, 8 to 10 months old per "variety-population" and per isolate were inoculated. For each population-variety, a control lot of 16 plants was inoculated with sterile water. Another constant control is made using a population-variety known for its susceptibility, inoculated with the reference isolate CAB003, so as to quantify the variation due to environmental conditions.

Analyses are based on the Generalised Linear Model (Annex 3). This model allows some variation in the number of plants per treatment.

Three trials were installed.

Trial 1

Four isolates: CAB003, OUG008, RDC002, and H2O
Two population-varieties *C. canephora* Guinean group: 1143 and 1144
Two temperatures: 25° and 28° C
16 plants per combination of treatments
Age of plants: 9 months
Symptoms readings made at 26, 50, 70, 105, and 162 days after inoculation.

Trial 2

Five isolates: CAB003, OUG008, RDC2, and OUG036. On water control (H2O)
One population-variety *C. canephora* Guinean group: 1143
Two temperatures: 25° and 28° C
16 plants per combination of treatments
Age of plants: 9 months
Symptoms readings made at 37, 58, 90, and 106 days after inoculation.

Trial 3

Four isolates: CAB003, OUG008, RDC002, and H2O (water, control)
One population-variety *C. canephora* Guinean group: 1151
Two temperatures: 25° and 28° C
16 plants per combination of treatments
Age of plants: 9 months
Symptoms readings made at 25, 50, and 100 days after inoculation.

1.2 Results

1.2.1 Inoculation technics

Trial 1

Table 1: Comparison of average mortalities, for two populations of 16 plants, 162 days after inoculation with 4 isolates

	Mortality %		Group (*)		
Isolate	**25°C**	**28°C**	**a**	**b**	**c**
CAB003	56	28	X		
OUG008	31	3		X	
RDC2	3	3			X
Control H2O	0	0			X

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05).

The effect of the population is not shown here, as it was not significant. The temperature, and isolate effects were significant, but not interaction could be found between the factors.

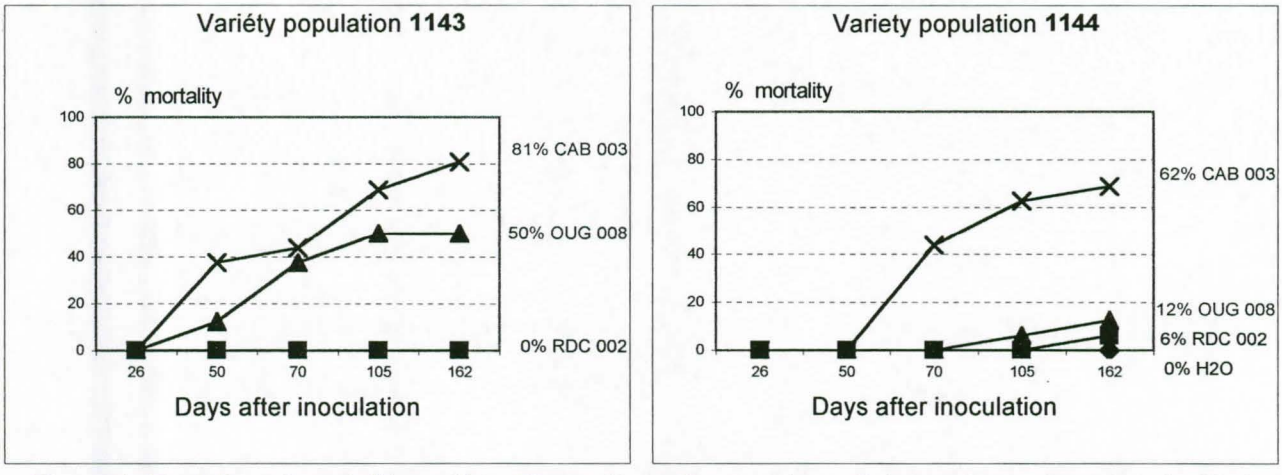


Figure 1: Evolution of mortalities within 32 seedlings *C. canephora* Guinean Group inoculated with 3 *Fusarium sp.* Isolates at 25°C

In population 1143 the first casualties occur after 50 days both at 25 and 28°C. The higher mortality is reached in all cases with CAB003. RDC002, as the water control, caused no mortality.
In population 1144 mortality occurs only after 70 days. CAB003 remains the most pathogenic. As for population 1143 the average mortality rate is higher at 25°C than at 28°C, especially for CAB003 and OUG008. RDC002 is responsible for 6% mortality only.

Trial 2

Table 2 gives the mortality rates observed.

Table 2: Comparison of average mortalities, within 16 plants of *C. canephora* Guinean Group, population 1143, 106 days after inoculation with 4 isolates

	Mortality %		Group (*)		
Isolate	**25°C**	**28°C**	**a**	**b**	**c**
CAB003	56	12	X		
OUG036	56	0	X		
RDC002	44	0	X	X	
OUG008	18	0		X	
Control H2O	0	0			X

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05).

Statistical results show a significant effect of temperatures and of isolates. No interaction was significant.

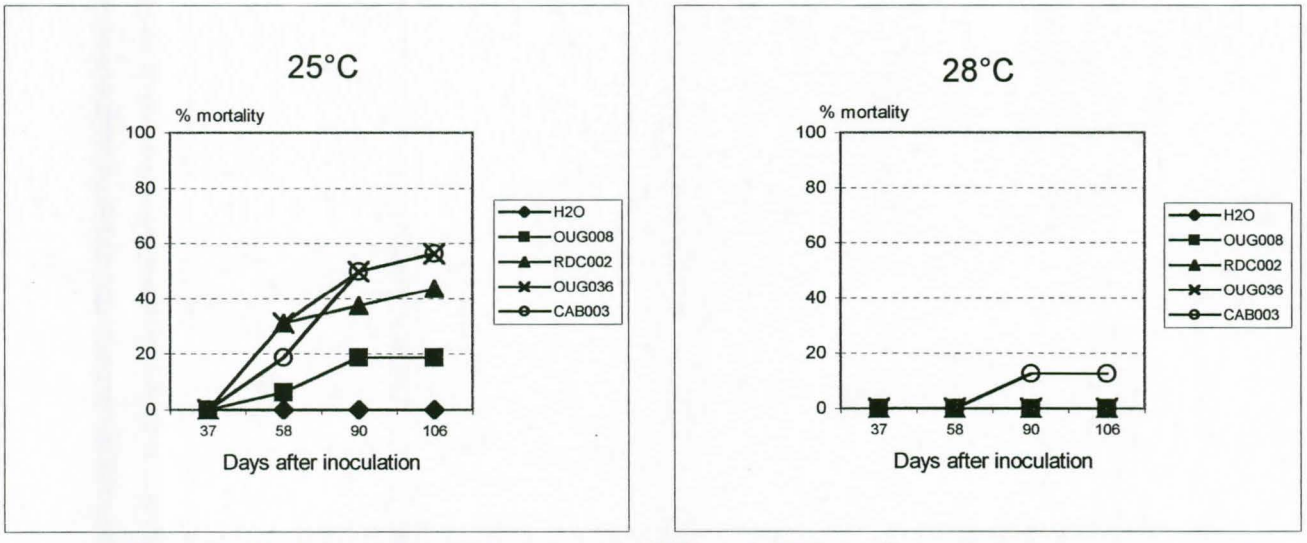


Figure 2: Evolution of mortality rates at 25°C and 28°C in *C. canephora* population 1143 (Guinean group) inoculated with 4 *Fusarium sp.* isolates

The first deaths were observed after 59 days at 25°C, and 90 days at 28°C.
At 25°C, and 90 days after inoculation, CAB003 and OUG036 were responsible for the highest mortality rates (50%), followed by RDCC002 (40%) and OUG008 (18.75%), and the control (water, no mortality).

Trial 3

Statistics indicate a significant effect of temperatures and of isolates and the absence of interaction.
The first dead plants, at 25°C, were observed for the 3 isolates after 50 days. At 28°C, it took 100 days. For all isolates, the highest death rate was obtained at 25°C after 100 days (Table X, Figure X).

Table 3: Comparison of average mortalities, within 16 plants of *C. canephora* Guinean Group, population 1151, 90 days after inoculation with 4 isolates

	Mortality %		
Isolate	**25°C**	**28°C**	**Group (*)**
CAB003	50	18	a
RDC002	32	12	a
OUG008	25	6	a
Control H2O	0	0	-

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05).

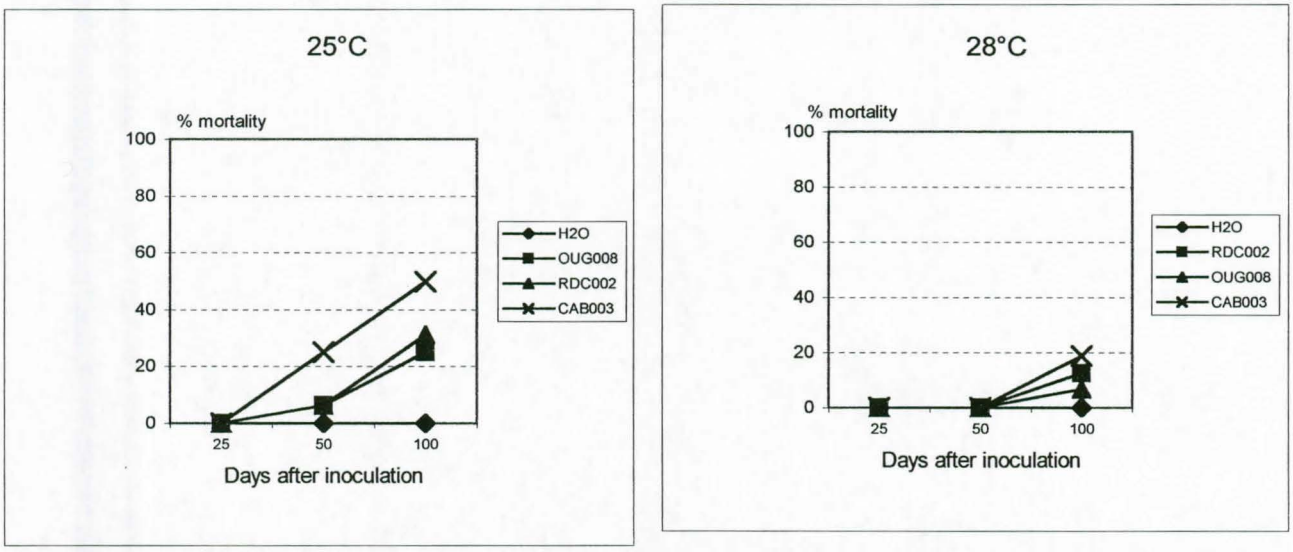


Figure 3: Evolution of mortality rates at 25°C (left) and 28°C (right) in *C. canephora* population 1151 (Guinean group) inoculated with 3 *Fusarium sp.* isolates

3.2.3 Evaluation of the variability in isolate aggressiveness using standard inoculation

A series of inoculations was made, using the protocol described earlier – syringe injection of a suspension of one isolate at 2 x 10^6 conidiae/ml in plants 8 to 10 months old. These trials were made at the same time as the tuning of inoculation techniques. Therefore some of the series were made at 28°C. All results are presented here.

Trial 4

Incubation temperature: 28°C
16 plants per replicate
Symptoms reading 100 days after inoculation

Table 4: Comparison of average mortalities, within 16 plants of 2 populations of *C. canephora* Guinean Group, , 100 days after inoculation with 4 isolates and a water control.

Plant material	Isolates			
	RDC002	CAB003	OUG008	RDC001
1143	12.50	6.25	0	0
1144	18.75	12.50	6.25	0
Mean	21.87	18.75	3.12	0
Group	a	a	a b	a b

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05). Water control: no mortality.

The range of the isolate is the same for both Coffee populations. The isolate RDC001 is no pathogenic in this trial.

Trial 5

Incubation temperature: 28°C
16 plants per replicate
Symptoms reading 120 days after inoculation

Table 5. Comparison of average mortalities, within 2 populations of *C. canephora* Guinean Group, , 120 days after inoculation with 4 isolates and a water control. 28°C.

Plant material	Isolates				
	CAB003	OUG033	OUG022	OUG036	OUG057
1143	25	18.75	18.75	12.5	0
1144	12.5	12.5	6.25	0	0
Mean	18	15	12.5	6.25	0
Groups	a	a	a	a b	b

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05). Water control: no mortality.

The range of the isolate is the same for both Coffee populations. Isolate OUG057 is no pathogenic in these trial.

Trial 6

Incubation temperature: 28°C
16 plants per replicate
Symptoms reading 114 days after inoculation

Table 6. Comparison of average mortalities, within 2 populations of *C. canephora* Guinean Group, , 120 days after inoculation with 4 isolates and a water control.

Plant Material	Isolates					
	OUG072	CAB031	CAB003	RDC002	OUG008	RDC001
1143	50	37.5	25	12.5	12.5	12.5
1144	25	18.75	31.25	12.5	12.5	0
Mean	37.5	28.12	28.12	12.5	12.5	6.25
Groups	a	a	a	a b	a b	b

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05). Water control: no mortality

The range of the isolate is the same for both Coffee populations. There is no significative difference between OUG057, CAB031 and CAB003, but in this trial the isolate CAB003 is not the more aggressive. The isolate RDC001 is slightly aggressive.

Trial 7

Incubation temperature: 25°C
16 plants per replicate
Symptoms reading 100 days after inoculation

Table 7. Comparison of average mortalities, within populations 1151 of *C. canephora* Guinean Group, , 100 days after inoculation with 4 isolates and a water control. 25°C.

Plant Material	Isolates					
	CAB003	OUG036	OUG057	OUG072	OUG031	OUG033
1151	100	75	68.75	56.25	31.25	0
Groups	a	a b	b c	c	c	d

(*) Isolates followed by the same letter do not differ statistically (Chi2 test, p=0.05). Water control: no mortality.

The isolate OUG033 is no pathogenic at 25°C but it was aggressive at 28°C

Isolate aggressivness

Tab 8 General and preliminary evaluation of the aggressivness of some isolates (to be confirm)

Population	CAB003		RDC001		RDC002		OUG008		OUG022		OUG031		OUG033		OUG036		OUG057		OUG072	
	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°	25°	28°
1143	+++	+		+	++	+		+		+				+	++	+	-	-		
1144	+++	+		-	++	+		+		+				+	-	-	-	-		
1151	+++	+			++	+	+	+			++	-	-	-	+++	-	+++	+++	++	++

+++ high aggressivness
++ intermediate aggressivness
+ low aggressivness
- no symptoms

A preliminary evaluation of the aggressivness of isolates is indicated in table 8. These results will must be confirm.

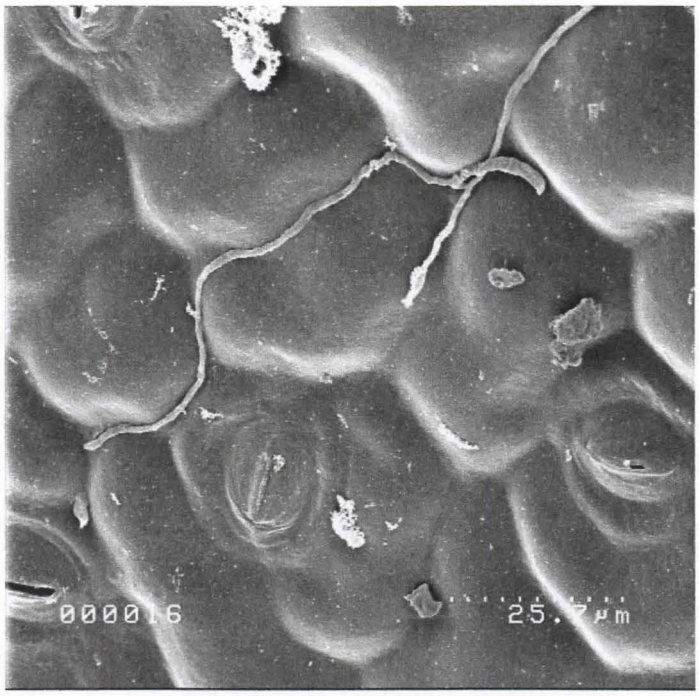


Fig 1 Germling tube 24 hours post inoculation on the inferior basis of a leave

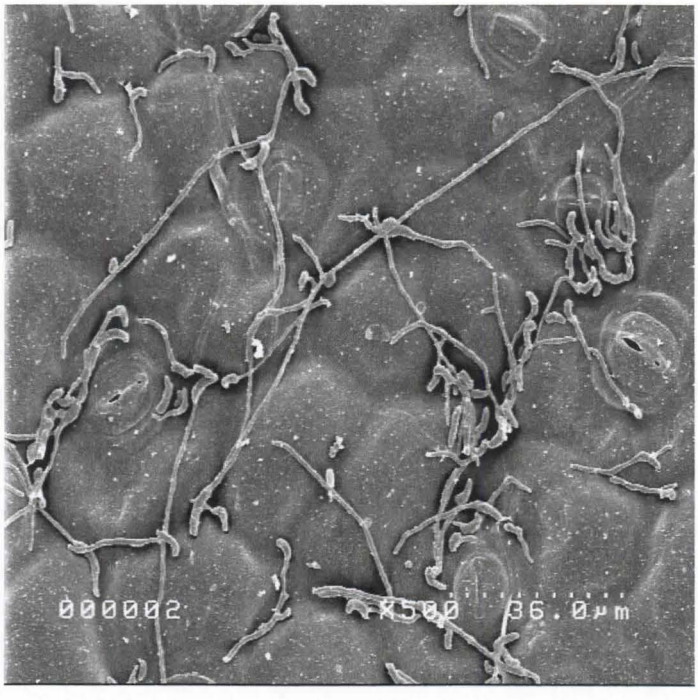


Fig. 2 Hyphal network 48 hours post inoculation. No infection hyphae or appressorium are visible

Task 3 Histo-cytological observation of the compatible-incompatible interaction.

Detailed information on the infection process, from penetration until spreading of the pathogen in host tissue are very important, especially at the early stages, because one objective is to develop a bio test on leaves. The inoculation techniques, dipping the root or injection by syringe are destructive. It's not possible to repeat the inoculation and the plant is destroyed.
The first aim of these studies is to observe the development of the hyphae on the surface of a leave without wounding by Scan Electron Microscopy (SEM)

The second aim is to describe the tissues colonisation in a whole plant at different stages of the infection and "atypical" symptoms observed during the infection process by artificial inoculation.

Entire leaves

Entire leaves of different *Coffea* species, a susceptible *C. canephora*, *C. arabica* and *C. liberica var. Excelsa* were inoculated with a droplet of a spore suspension (CAB003) adjusted to 1.10^6 conidiae/ml on the lower side of the leaves, without wounding

24 hours after inoculation, germination has occurred, and mycelium starts spreading on the leaf area (Fig. 1). It follows an intra-cellular furrow. There is no evidence of the presence of any appressorium-like penetration structure.

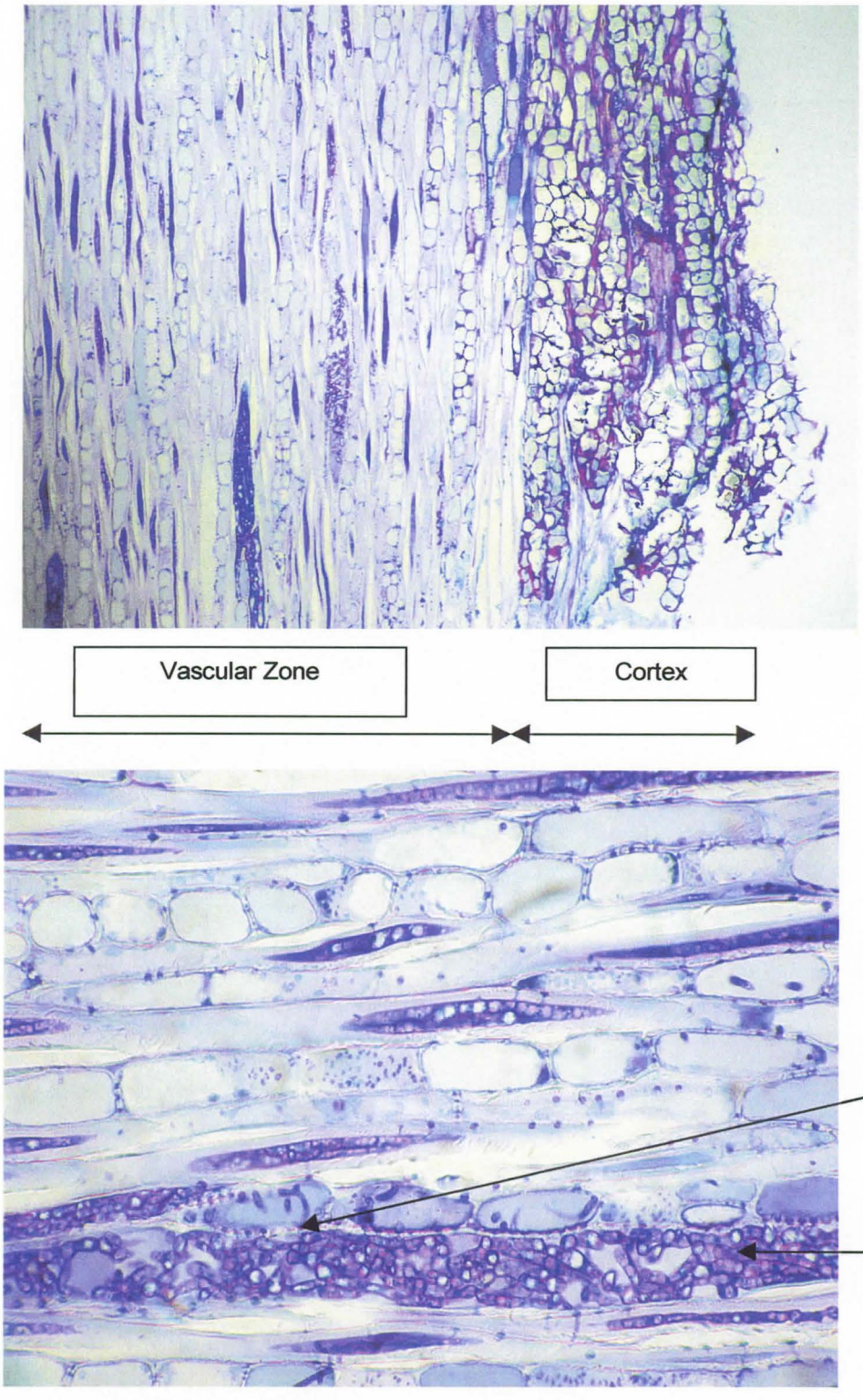
48 hours after inoculation, whatever the Coffee species, mycelium continues spreading over the leaf area (Fig 2). Although it passes a stomata it does not use it as a way for penetration.
The mycelium web continues getting denser up to 96 hours without developing any mean or structure of penetration.
Macroscopic observations after 10, 20, and 35 days revealed no symptoms. Light microscopic observation of thick sections confirmed the absence of mycelium inside the tissues.

Leaf disks

After 24 hours observations are made at the edge of the leaf disk in the wounded zone. Mycelium can be seen in the lacunas of the spongy mesophyll. The presence of polyphenols witnesses the response of the cells to the aggression.

After 8 days one can observe deformations of the cells walls, early sign of their degradation. Colonisation of the tissues is both inter and intracellular.

After 35 days the cells walls are strongly deteriorated, and many completely phenolised cells can be seen. Paradoxically, no symptoms can be seen on the leaf area.



Figs 3 - 4 - Hyphae colonisation of the vascular zone 45 days post inoculation of a 18 months old plant

Entire plants aged 18 months

The collection was made 3 cm above the injection point, 45 days after inoculation. First characteristic wilt symptoms could be seen (Figs 3-4).

Light microscopic observation indicates that the hyphae have colonised the whole xylem and phloem vessels. Two types of mycelium are observed. One, 4 to 5 µm diameter is present mainly in the phloem tube, the other one is smaller, and inside the cells. The mycelium density inside the vessels is very high, and obviously it blocks water and sieve transportation. Sometimes conidia can be seen in the vessels.

No mycelium could be found in the apex part of the plant, but the cells were deteriorated.

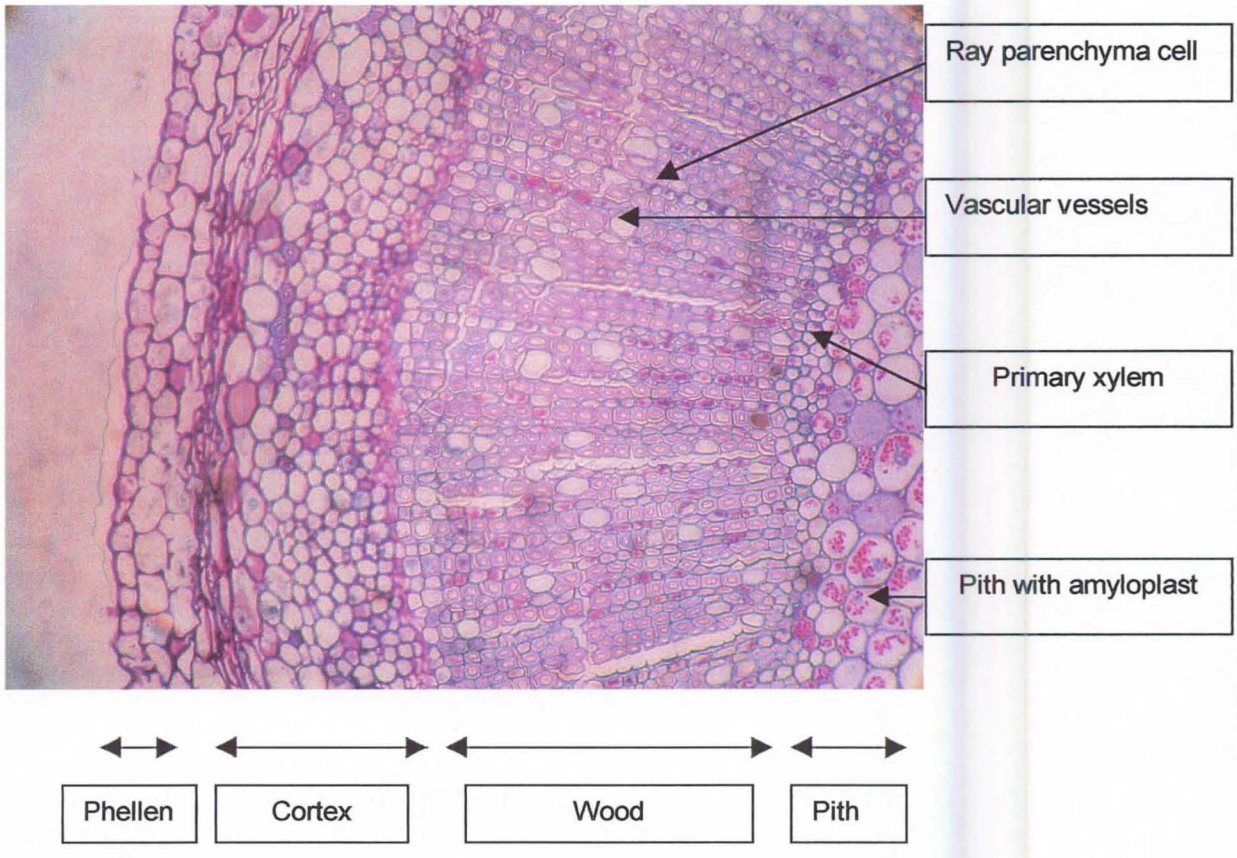


Fig 5 - Cross section through a health stem

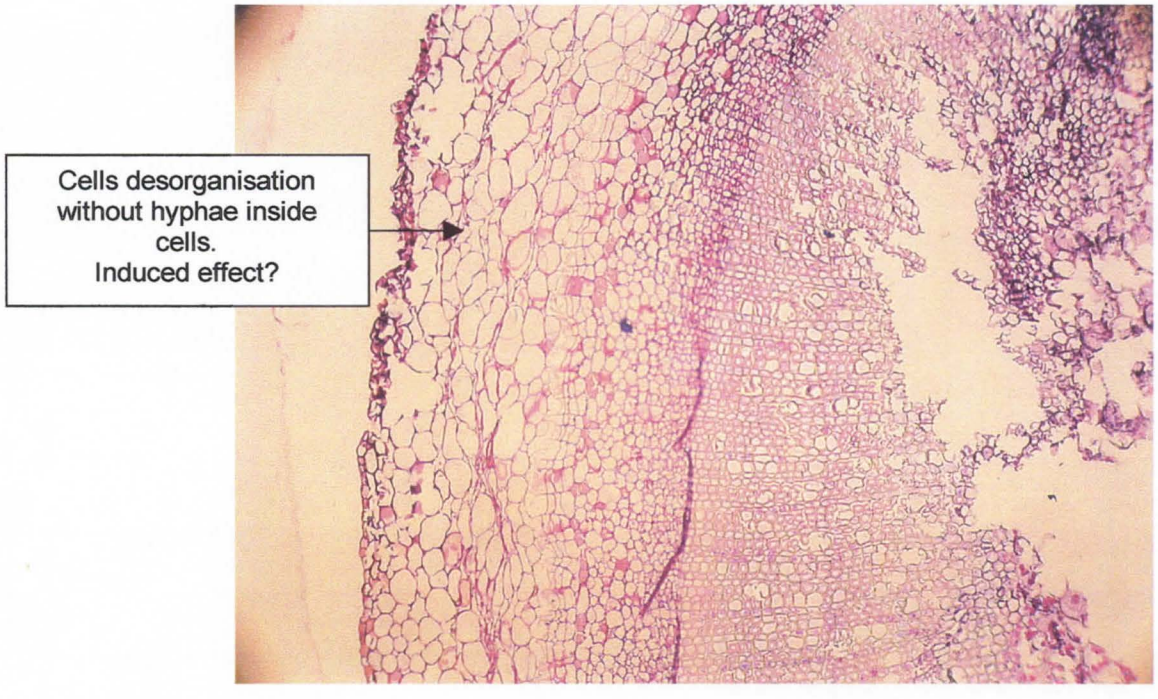


Fig 6 - Cross section trough the 3rd internode of an infected stem 1144 (9 old months plant) with CAB003, 60 days post inoculation. No mycelium is observed

9 months old plants

In this study, with light microscopic observation 2µm thick sections were cut (Figs 5 - 6)

A compatible couple *C. canephora* (Population 1143) – Isolate CAB003 was used.
Cytological observations were done at the earliest symptoms stage when the leaves at the top of the plant start wilting, 8 weeks after inoculation. The five internodes were sampled starting from the cotyledonnaries leaves.
Light microscopic observation at the level of the first internode show that epidermis and external parenchyma have disappeared. The cork and the phellogen have been partially deteriorated, which causes a slight disorganisation of this tissue. The wood tissues are disturbed in some places, however as a whole they are globally not much deteriorated. No trace of mycelium could be seen on these cuts.

At 2nd internode level epidermis, external parenchyma and phellogen are missing. The parenchyma cells are distorted and slightly hypertrophied. The xylem structure is intact as well as its cells, and no mycelium is found.

At 3rd internode level, epidermis is missing. One can notice hypertrophy of external and upper parenchyma cells that leads to a clear deformation of these tissues. Some regions are even strongly deteriorated. Furthermore many cell walls are re-cloisonné. Merely a few pieces of the phellogen layer are still present. Xylem and pith cells are strongly deteriorated. They contain a large quantity of starch. No mycelium is found.

At 5th internode level epidermis is missing. External parenchyma cells are strongly hypertrophied, which causes a clear modification in the tissues' structure. The phellogen is missing. No mycelium is found. A significant accumulation of starch can be observed in pith as well as in wood's lower part cells.

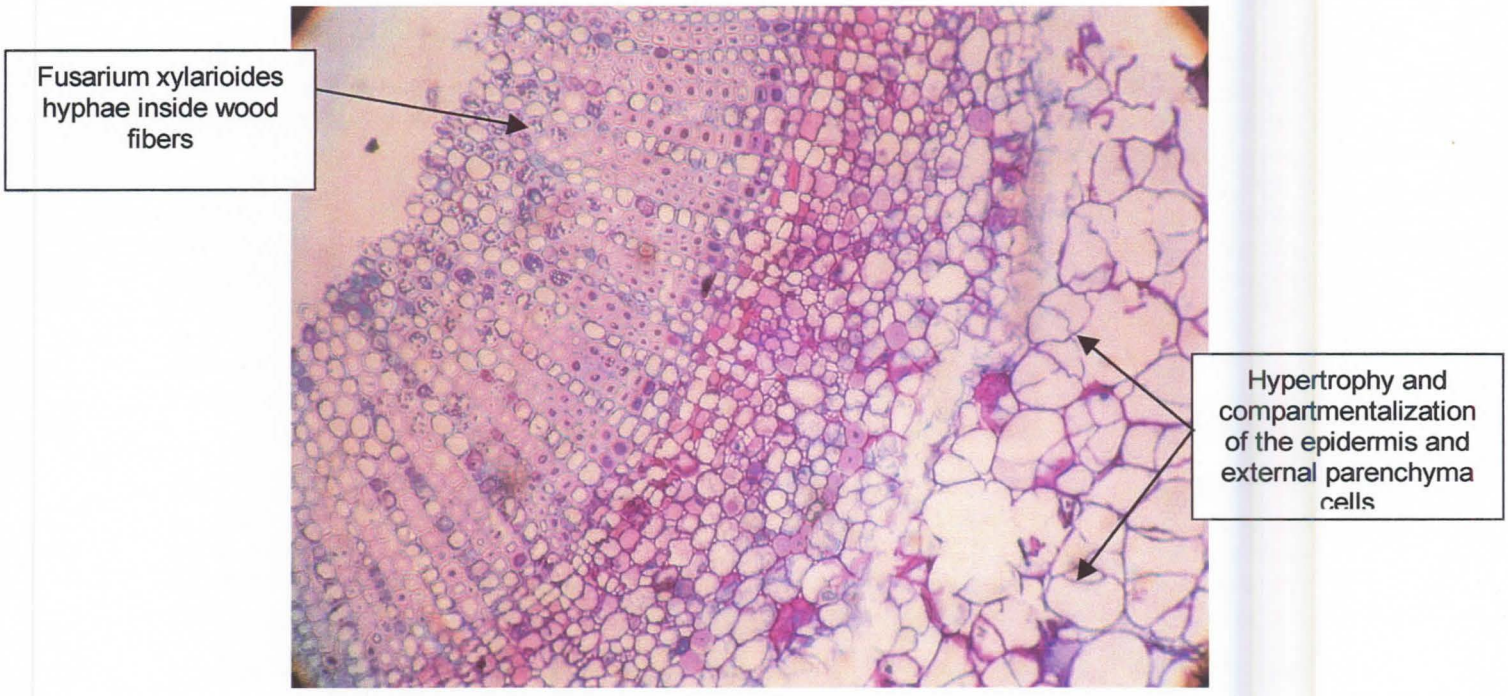


Fig 7a

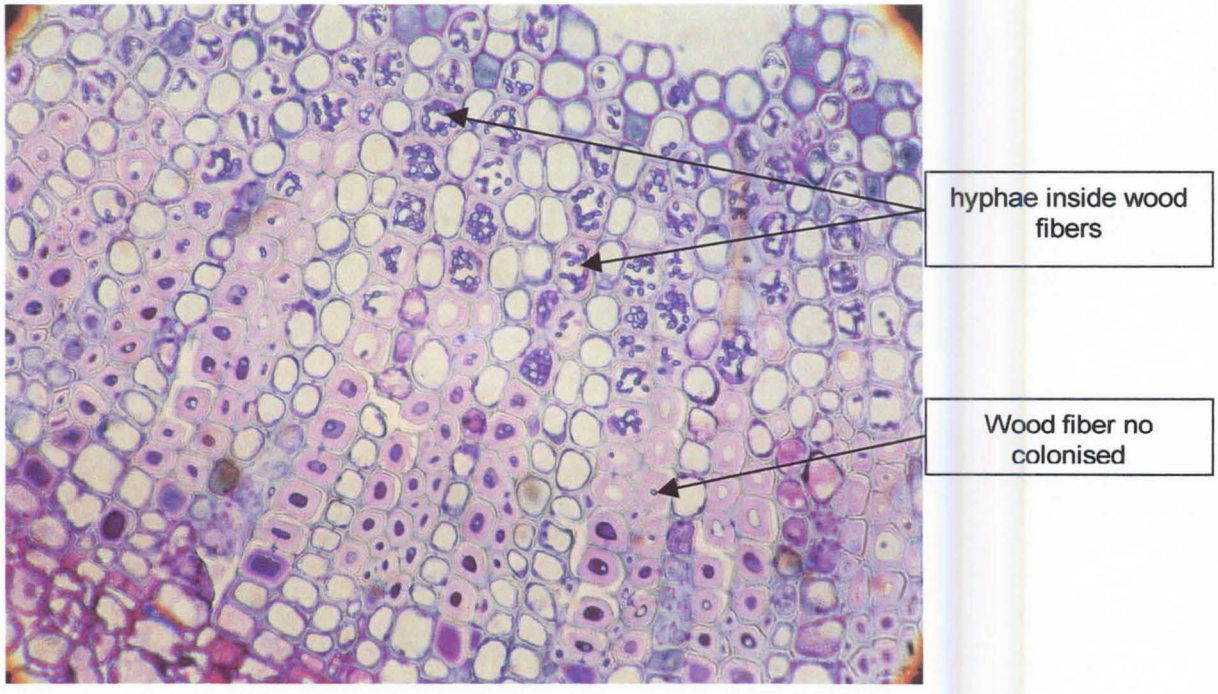


Fig 7a & 7b: Cross section in a wart-like structure in an infected stem 1143, 9 months old plant with RDC2 isolate.

Observation of "atypical symptoms"

Coffee Population No. 1143 aged 9 months was inoculated with Isolate RDC002.
At the surface of the stem, and at the level of the fore last internode, one can observe a "wartlike structure" due to the tissues' deformation. This slightly discoloured outgrowth spreads over about 1 cm along the stem. It seems to be made of small under-epidermic clusters.

At first one can observe a hypertrophy of the epidermis' and external parenchyma's cells. This results into an increase of the vacuoles volume of these cells, and into the disorganisation of the tissues. The normal structure of the parenchyma has disappeared, and some of the cells have been compartimentalized. The cells of phellogen are deteriorated..
The structure of the cork cells has remained intact, with a slight hypertrophy of nuclei of the cells.
The libero-ligneous layer cannot be seen.
The fungus' presence is witnessed in wood tissues, more precisely in fibres. It appears as a blue, intracellular mycelium that colonises the fibres linearly following the rays.
The wood fibres that are colonised are the closest to the pith.
One notices that the cells that contain mycelium have no, or very few lignin on their walls.
Vessels do not seem to be colonised by mycelium.

CONCLUSIONS

Standard inoculation technique for routine screening

The validation of the trials are validated by the presence of constant control Coffee genotypes 1143 or 1151 inoculated with CAB003. This controls indicates the variation due to the experimental conditions or to the physiologic state of the plants, depending on age and on vigour. For all these trials the death rates for the control vary from 25 to 83%. The reason for this variation is not clear, however it may be due to variation in age and vigour of the plants. As a whole the tests seem to be repeatable.
Due to the low quantities of seedlings available not enough replications could be made.
However we were able to achieve 2 replications in the time for 9 population-varieties with 2 isolates. Despite a variation in the results no noticeable change in the range is observed except for population 1178 that looked almost resistant in one case (16% dead) and very susceptible in the other case (92% dead). For these 2 series the same variation, in the same proportion was observed for isolate RDC002. This variation may be due to the physiological stage of the seedling (age).
The systematic use of a water control in all trials is a proof that the technique used does not harm the plants and has no influence on the expression of symptoms. Therefore in the future this water control will not be made any more, as it uses too many seedlings.
In any case this result shows the influence of environmental condition, especially temperature, on the experiment and on the expression of symptoms. This implies to introduce a control in every trial, so as to ascertain the validity of the trial over time.

This result may be explain by the two hypotheses below:

- either the temperature 25°C is more conducive to the fungus development (see chapter X), then tissue colonisation and pathogen aggressiveness may be reinforced,
- or the optimal temperature for coffee development is 28°C. Placing the coffee plants at 25°C creates conditions slightly stressing, making defence mechanisms less efficient.

A valid model is necessary to analyse the results. The generalised linear model look appropriate for that purpose.(Annex 4)
The standard inoculation technique retained at this stage is syringe inoculation of a calibrated 1 x 10-6 conidia/ml suspension in 8 to 10 months old plants, that are placed at 25°C with a 12/12 hours photoperiod for incubation.

The detailed protocol can be seen in Annex 3

Isolate aggressivness

Trails 4 to 6 were made at 28°C, before the protocol was definitely decided. Thus the results are probably lower than what could have been expected at 25°C. For instance isolate OUG057 produced no symptoms at 28°C on Populations1143 and 1144, but induced 68% mortality on Population 1151 at 25°C. Also the mortality observed with isolate OUG036 is much lower at 28°C than at 25°C.

On the other hand isolate OUG033 produces symptoms at 28°C on Populations 1143 and 1144 (Trial 5) but no symptoms on Population 1151 at 25°C (Trial 7).
This result also poses the question whether the isolates' behaviour depends on temperature, or is it a differential reaction? Further investigation is required to answer the question.

Isolate RDC001, as others, has a quite variable production of conidia (sometimes no production at all). It is thus considered as non pathogenic.

CAB003 in these trials is always seen as the most pathogenic isolate.

The variability in pathogenicity is confirmed especially in Trial 6 and 7 where a ranking in mortality rates can be seen. As conclusion, a variation like a continuum in aggressivness of the different isolates tested is observed. These, will must be confirmed.

In experimental condition, the temperature influence significativly symptoms intensity and percentage of mortality.

Characterisation of the infection process

Leaves

The observation of the leaf area shows that *Fusarium xylarioides* does not develop any appressorium-like penetration structure on the leaf area, and that stomata are not use as ways of penetration of the fungus.
However if the tissues are wounded mycelium penetrates and develops by colonising the cells of the spongy mesophyll. No external symptoms indicate the fungus' presence inside the tissues.
A wound is thus absolutely necessary for infection to start. But, the absence of visible symptoms makes the use of leaf- or leaf disk- test impossible.

18 months old entire plant

In the case of 18 months old plants mycelium invades xylem and phloem, and develops a dense hyphal colonisation that are thought to block water and sap transportation. The cells as well are invaded and deteriorated. Inside the plant, the fungus seems to develop inside the vessels, but also through the intercellular space.
However, cuts at various internode levels of plants that exhibit the early symptoms of the disease do not allow localising the fungus. Either the cuts were made in zones where the fungus was absent despite early symptoms, or mycelium had not reached that level of the plant.
This fungus may act by blocking the water and sieve fluxes in xylem and phloem fibers. One point to strengthen this hypothesis is that, in many cases, suckers develop below the inoculation point. However, in most cases these suckers become sick rapidly, and do not survive.

9 months old entire plant

In this case on a plant with apparent symptoms, whatever the internode level, no mycelium was found inside the tissue, but the cells and the structure was partially deteriorated. Remote reaction of these cells to a stress induced by a mycotoxine may be envisaged

Atypical symptoms "wartlike" structure

On a plant without apparent symptoms, 3 nodes away above the infection point, we observed a "wartlike" structure. We were able to trace mycelium filaments.

The presence of mycelium, 45 days after infection, at a 3 nodes distance of the infection point, indicates that it migrates insides the tissues; either through hyphae elongation, or through translocation of conidia that develop occasionally, and are transported by sap or by water towards the apical part of the plant.

Several times indicators of an aggression were observed, namely degradation of the cellulosic cell walls, or compartmentalization of the cells. However no mycelium was found outside these cells.

The latter hypothesis deserves further investigation. Indeed if a mycotoxine takes part in the infection process, then a non-destructive test based on the use of that mycotoxine may be developed.

WP3: Breeding for resistance

Task 3 Conduct screening tests using isolates with a wide range of aggressivness

These first tests at Cirad were started in order to validate the inoculation technique at a wider scale, and to look for resistance factors within a wide representation of the 5 identified genetic groups within the *Coffea canephora* species.

As a reminder the tests were conducted on seedlings from free-pollinated seeds but most of them collected on well-identified coffee trees/clones. In the text they will be designated as "progeny". Indeed their mother parents are clones or cloned entries from the wild, which are known for the group they belong to. Their group is indicated in Table 1. A total of 5 trials were undertaken on 8 to 10 months old plants.

The protocol was similar to the one described in the Chapter "Elaboration of a standard inoculation technique", and analyses were made using the General Linear Model.

Table 1: Description of 5 experimental protocols to conduct screening tests at Cirad

Trial	Population	Isolates	T°	Days post inoculation	Nb of plants
8	1143	CAB003 - RDC002	25°	106	12
	1158				
	1162				
	1167				
	1172				
	1173				
	1177				
9	1143	CAB003 - RDC002	25°C	120	12
	1169				
	1181				
	1184				
	1186				
	1189				
	1208				
10	1143	CAB003 - RDC002	25°C	97	12
	1164				
	1178				
	1183				
	1187				
11	1151	CAB003 - RDC002	25°C	88	12
	1157				
	1158				
	1162				
	1167				
	1172				
	1173				
	1177				
	1178				
	1186				
12	1143	CAB003	25°C	120	16
	1153				14
	1160				9
	1191				14
	1197				17
	1213				12
	1215				17
	1219				14
	1227				11
	1235				13
	1236				7

Results

Results of the 5 trials are summarised in Table 2

Table 2: Mortality rate of *C. canephora* progenies in 5 trials after inoculation with two *F. xylarioides* isolates

Trial	Population	CAB003	RDC002	Mean	Group(**)
8	1143	83	42	62.5	A
8	1167	75	42	58.5	A
8	1162	67	50	58.5	A
8	1177	58	50	54	A
8	1158	75	42	42	A
8	1173	0	16	16	B
8	1172	0	0	0	B
Mean		*51*	*34*	*28*	
9	1186	75	42	58.5	a
9	1208	50	42	46	a
9	1181	33	33	33	ab
9	1184	33	16	24.5	ab
9	1143	25	16	20.5	ab
9	1189	16	16	16.5	b
9	1169	33	0	16	c
Mean		*38*	*24*	*32*	
10	1164	50	25	37.5	a
10	1183	25	50	37.5	a
10	1187	42	16	29	a
10	1143	25	33	29	a
10	1178	16	16	16	a
Mean		*32*	*28*	*25*	
11	1162	100	92	96	a
11	1177	84	100	92	a
11	1178	92	92	92	a
11	1186	84	84	84	ab
11	1167	100	67	83.5	ab
11	1151	66	66	75	ab
11	1158	72	50	66.5	b
11	1157	66	58	62	b
11	1172	42	42	42	b
11	1173	8	0	4	c
Mean		*71*	*65*	*68*	
12	1213	92	-	-	a
12	1153	86	-	-	ab
12	1191	79	-	-	abc
12	1236	71	-	-	abcd
12	1215	65	-	-	abcd
12	1227	64	-	-	abcd
12	1235	54	-	-	bcd
12	1143	50	-	-	cd
12	1197	35	-	-	de
12	1219	14	-	-	e
12	1160	11	-	-	e
Mean		*56*			

(*) Reading: days after inoculation
(**) Means followed by the same letter are not significantly different (Newman & Keuls p=0.05)
(-) No record

The isolate effect was never significant.

Trial 8

Mortality rate varies from 0 to 83%. The isolate effect is not significant, but the average rate caused by RDC002 (34%) is lower than by CAB003 (51%). Populations 1172 and 1173 seem resistant, which has to be confirmed.

Trial 9

Mortality varies from 0 to 75%. The mortality rate for Control population No.1143 with both isolates is lower than in trial 8. Some interaction can be seen between Population 1169 and both isolates as its mortality rate is 33% with CAB003, and 0% with RDC002 thus, unlike for others, lower. This will have to be confirmed.

Trial 10

No significant difference can be found between isolate or between populations

Trial 11

The isolate effect is not significant. Mortality rates vary from 0 to 96% for the most susceptible population, No. 1162.
Comparison between Trials 8 and 11 show that for 3 populations namely 1162, 1167, and 1172 mortality is slightly higher in Trial 11 than in Trail 8, for 1158 it is stable, and for 1173 as well Trial 11 confirms the resistance found in Trial 8.
Figure 1 compares the means for each isolate. It shows no evidence of isolate interaction with the progenies concerned.

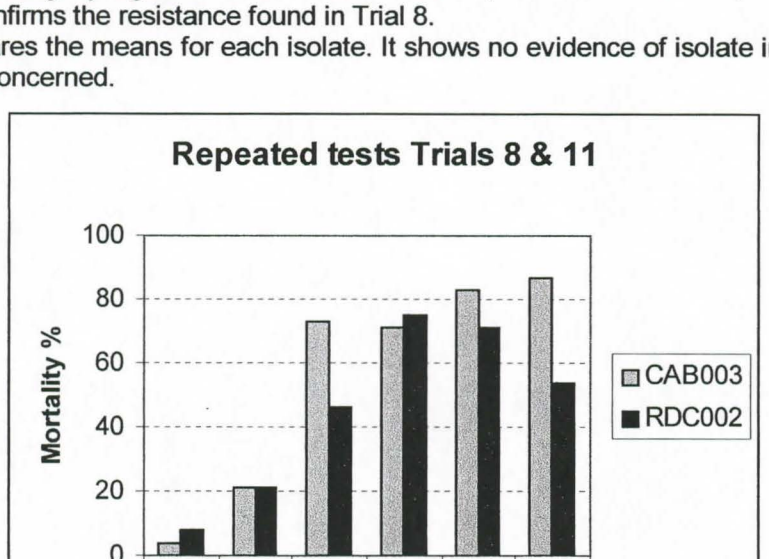


Figure 1: Reaction of six progenies to two isolates in two replications in time

Trial 12

Mortality varies continuously from 11% to 92%. For Population 1197, the mortality rate (35%) confirms the result in trail 10.

Conclusions

Methodology

Note that control Populations No. 1143 or 1151 have been used in each trial, and inoculated with isolate CAB003. These control validate the trials. Besides, it allows controlling the variations in symptoms intensity due to experimental conditions or to physiologic stage of the plants, namely age and vigour in particular. For that series of trials the average mortality ranges from 25 to 83%. The cause of this variation is not well known, but variation in age and vigour may be one reason.

The results as a whole indicate a rather good repeatability. The lack of seedlings did not allow a sufficient number of replications at different dates. Thus no statistical analysis can be done.

For 9 Progenies (trial 9 and 11) two inoculations could be done at different dates.
One notices some fluctuation in mortalities, but, as a whole, no inversion in range could be seen, except for Population 1178. In a first experiment it may have been considered resistant (16%) whereas in the second one it looked very susceptible (92% mortality). The same variations was noted with isolate RDC002 thus the variation may be due to the physiologic stage of the plants.

The systematic use of a water control in all trials showed that the technique itself had no influence on the expression of symptoms. Therefore this water control will not be used any further.

Evaluation of susceptibility

This first series of tests used *Coffea canephora* populations that can be considered representatives of the five genetic groups identified within the species (Table 2).

At a first glance, with isolate CAB003, these populations express various levels of resistance.

Subject to confirmation, Populations 1160, 1173, 1219, and 1230 look resistant to *F. xylarioides*.

The variation in reactions to the disease vary continuously from 0 to 100%, which is especially conspicuous in Trial 12.

For some Populations the response is clear, namely 1213, 1153, 1191, 1236 (susceptible) or 1219 and 1160 (resistant). For others the rate is intermediate, around 50%. Limits between these intermediate levels of resistance cannot be defined yet. These results can be considered a trend, and will have to be confirmed. However, it can be noted that some genotypes have already be identified as possible sources of resistance.

Table 3: Summary of mortalities per progeny, per isolate, and per trial, Trials 8 to 12.

			Isolate	
Progeny	**Mother parent: origin**	**Trial**	**CAB003**	**RDC002**
1143	Clone 119	9	25	16
1143	Clone 119	8	83	42
1143	Clone 119	10	25	33
1143	Clone 119	12	50	
1151	Clone 529	11	66	66
1153	liberica liberica	12	86	
1157	canephora Nana	11	66	58
1158	canephora Nana	8	75	42
1158	canephora Nana	11	83	50
1160	canephora Nana	12	11	
1162	Congolais	8	67	50
1162	Congolais	11	100	92
1164	Congolais	10	50	25
1167	Kouilou	8	75	42
1167	Kouilou	11	100	67
1169	Kouilou	9	33	0
1172	Guinean	8	0	0
1172	Guinean	11	42	42
1173	Guinean	8	0	16
1173	Guinean	11	8	0
1177	Clone 197:	8	58	50
1177	Clone 197:	11	84	100
1178	Clone 197:	10	16	16
1178	Clone 197:	11	92	92
1181	Clone 200:	9	33	33
1183	Clone 202:	10	25	50
1184	Clone 202:	9	33	16
1186	Clone 400:	9	75	42
1186	Clone 400:	11	84	84
1189	Clone 503:	9	24	16
1191	Clone 503:	12	78	
1197	Group C	10	42	16
1197	Group C	12	35	
1208	Group: SG2	9	50	42
1213	Group: SG2	12	92	
1215	Guinean	12	65	
1219	Guinean	12	14	
1227	B	12	64	
1235	C	12	54	
1236	C	12	71	

Grey: Replicated

UCL

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

First Annual Report (November 2001 to October 2002)

Université catholique de Louvain
Unité de Phytopathologie
Croix du Sud 2bte 3
B-1348 Louvain la Neuve - Belgium

COWIDI-ICA4-2000-10312

Report 01/11/2001-31/102002

Partner 2 Université catholique de Louvain (UCL)

Prof. H. MARAITE attended on 4-7 March 2002 the First General Meeting of the Project at Kampala, Uganda. At this meeting it was agreed that UCL will focus on the mechanisms of variation of the coffee wilt pathogen *Gibberella xylarioides* through the sexual and the asexual cycle, in close cooperation with CABI and CIRAD who are characterizing the diversity at mycological and pathogenic level, respectively, as well as with CORI and UNIKIN, involved among others in studying the life cycle and the pathogen-coffee genotype interactions *in situ.* Knowledge on the mechanisms and possibilities of variation is indeed of key importance for selection of coffee genotypes with stable resistance to *G. xylarioides.* It was also agreed that UCL would assist UNIKIN in characterization of the fungi associated with coffee wilt symptoms in DRC by crosschecking isolations from samples collected in DRC and providing mycological training to UNIKIN scientists during stays at UCL.

As a follow up of this meeting, a review of "The life cycle of *Gibberella xylarioides* Heim & Saccas" (annex 1) as well as instructions for "Monitoring of perithecia and conidia production of *Gibberella xylarioides* on naturally infected coffee trees"(annex 2) were prepared and dispatched to the other partners.

Because of the budget allocated to UCL allowed only a 3 year grant to a PhD student within the 4 year period of the project, and seen the necessity to first gain more information on the pathogen diversity as well as on sporulation *in situ*, recruitment of the PhD student Pascale LEPOINT, MS in agronomy, has been postponed to November 1, 2002.

The contributions to the variousWP's and Tasks during the reporting period are detailed below.

WP1 Pathogen diversity

Task 1: Collection of both forms of the fungus on various parts of the trees, possibly on alternative hosts, in affected regions

Analysis by UCL of samples collected in Africa

1. Samples provided by UNIKIN (Tabel 1)

Sample identification	Sampling date	Location	Variety/ clone	Origin	Area	Country
J.E.5	12/03/2002	stem base	Robusta	trial	Kinshasa	RDC
Lemba 1	12/02/2002	stem base	Kwilu	farmers field	Bas-Congo	RDC
Kakongo 1	13/02/2002	stem base	Kwilu	farmers field	Bas-Congo	RDC

Dispatched on 12/03/2002 by A. KALONJI-MBUYI
Analysed on 17/03/2002 by H. MARAITE

- J.E.5, subsample A showed a deep injury up to the xylem. The numerous perithecia developing on the injured tissues appeared to be of *Glomerella* sp. or *Physalospora* type. Isolation on water agar from the tissues below these perithecia with hyphal tip transfer to SNA after one week. Typical isolate: HM170302-12D
J.E.5, subsample B also showed an injury up to the xylem and abundant perithecia and pycnidia on the surface. Isolations were performed from discoloured xylem vessels, the vascular symptoms bearing no direct connection to the injury.
Isolations yielded always strains with *Fusarium solani* characteristics, but never *G. xylarioides*.
Representative isolates: HM170302-13A and HM170302-14B.

- Lemba 1, subsamples A and B showed numerous pycnidia of *Botryodiplodia theobromae* also causing blackening of the bark grey discolouration of the wood.

- Kakongo 1 subsamples A and B also colonisation by *Botryodiplodia theobromae*. No detection nor isolation of *G. xylarioides*.

Conclusion

G. xylarioides has not yet been identified from the Kinshasa and Bas-Congo area of RDC.

2. Samples from Kituza, Uganda

During the visit on 06/03/2002 at the Coffee Research Centre, Kituza, Uganda bark samples from 5 diseased coffee trees were taken for analysis of the possible occurrence of mature perithecia for isolation of single ascospore strains.

Analysis by help of a microscope revealed only empty perithecia.
On 17/03/2002, H. MARAITE performed isolations on water agar or SNA. After a tentative identification hyphal tips from *G. xylarioides (*HM170302-15A, HM170302-16A, HM170302-19A, HM170302-20D) and *Fusarium* sp. (HM170302-18A, HM170302-20A) isolates were transferred on 24/03/2002 to SNA slopes and dishes. Single conidia subculturing and further characterization were performed by Dr Françoise Munaut of BCCM/MUCL.

Task 2: Identification, storage, and exchange of isolates.

Morphology and molecular characteristics were compared with *G. xylarioides* strain MUCL 14186. DNA was extracted from cultures grown on Malt broth. 28S as well as ITS1-ITS2 region were sequenced and compared with data available at EMBL (Table 2).

- Identification of the isolates HM170302-15A, HM170302-16A, HM170302-19A, HM170302-20D from material collected in Uganda as *G. xylarioides* has been confirmed.

- The 28S of the isolates HM170302-13A, from RDC and HM170302-18A and HM170302-20D from Uganda showed 100% homology with *F. falciforme*, and the ITS1-ITS2 region 98% homology with *Nectria haematococca.*

- Isolate HM170302-14B is still under investigation.

The original hyphal tip isolate as well as 2 single conidia strains are deposited at BCCM/MUCL (table 2).

Table 2. Accession number and identification of the Fusarium spp. isolates from coffee characterized at BCCM/MUCL in the frame of the COWIDI project in 2002.

Isolate n°	**MUCL access. n°**	**morphology**	**Identification by sequence homology**	
			28S	**rDNA**
JM12041[a]	14186	*F. xylarioides*	*F. xylarioides*	-
HM170302-13A[b]	43879	*Fusarium* sp. 1	-	-
13A-1	43880	*Fusarium* sp. 1	*F. falciforme* (100%)	*Nectria haematococca*(98%)
13A-2	43881	*Fusarium* sp. 1	-	-
14B	43882	*Fusarium* sp. 2	-	-
14B-1	43883	*Fusarium* sp. 2	in progress	in progress
14B-2	43884	*Fusarium* sp. 2	-	-
15A	43885	*F. xylarioides*	-	-
15A-1	43886	*F. xylarioides*	*F. xylarioides*	-
15A-2	43887	*F. xylarioides*	-	-
16A	43888	*F. xylarioides*	-	-
16A-1	43889	*F. xylarioides*	*F. xylarioides*	-
16A-2	43890	*F. xylarioides*	-	-
18A	43891	*Fusarium* sp. 1	-	
18A-1	43892	*Fusarium* sp. 1	*F. falciforme* (100%)	*Nectria haematococca*(98%)
18A-2	43893	*Fusarium* sp. 1	-	
19A	43894	*F. xylarioides*	-	
19A-1	43895	*F. xylarioides*	*F. xylarioides*	-
19A-2	43896	*F. xylarioides*	-	
20A	43897	*Fusarium* sp. 1	-	-
20A-1	43898	*Fusarium* sp. 1	*F. falciforme* (100%)	*Nectria haematococca*(98%)
20A-2	43899	*Fusarium* sp. 1	-	-
20D	43900	*F. xylarioides*	-	
20D-1	43901	*F. xylarioides*	*F. xylarioides*	-
20D-2	43902	*F. xylarioides*	-	

[a] Isolated and identified in 1960 by J.A. Meyer from *Coffea robusta* collected at Yangambi

[b] Original hyphal tip isolate and single conidia strains –1 or –2

-: not tested

Conclusion

Besides *G. xylarioides*, other bark and wood colonizing fungi may be associated with external and internal symptoms suggesting coffee wilt. This highlight the interest of combining field surveys with pathogen isolation.

The *F. falciforme* strains will be included in the pathogenicity trials in order to evaluate the status as primary pathogen or secondary colonizer of this species on coffee.

Task 3: Evaluation of the variability in isolate aggressiveness using standard inoculation tests

Start of pathogenicity trials are planned during year 2 in close collaboration with CIRAD.

Task 4: Description of the fungal life cycle, asexual and sexual phases.

UCL has prepared a review of "The life cycle of *Gibberella xylarioides* Heim & Saccas" (annex 1) as well as instructions for "Monitoring of perithecia and conidia production of *Gibberella xylarioides* on naturally infected coffee trees"(annex 2). These documents were dispatched to the African partners in April 2002.

Up to now no information on the application of these instructions nor samples bearing *G. xylarioides* perithecia have been received from the African partners.

Hopefully samples will soon be available. Other means for obtaining samples and single ascospore strains will be explored.
Strain crossing trials on artificial and natural media will be set up.

H. MARAITE

Annex 1
to the 2002 report of UCL

The life cycle of *Gibberella xylarioides* Heim & Saccas

H. MARAITE

Unité de Phytopathologie, Université catholique de Louvain, Louvain-la-Neuve

Sporulation

Teleomorph morphology and production

Black to violaceous perithecia, embedded single or in groups in dark purple stromata can often be spotted at the stem base of coffee trees showing typical wilt symptoms and the blackening of the wood. The stromata generally emerge from cracks in the bark. According to Heim & Saccas (1951) on *Coffea excelsa* in Ubangui and Meiffren (1957) on *C. canephora* in Ivory Coast, as well as Booth & Waterston (1964), the perithecia are globose to ovoid, thick walled, 200-400 x 180-300 µm. Perithecia corresponding to the latter description where also observed by Kranz & Mogk (1973) on wilted *C. arabica* trees in Ethiopia.

The asci are cylindrical, thin walled, shortly pedicellate, 90-110 x 7-9.5 µm (60-80 x 5.5-8µm according to Meiffren, 1957) with 8 monostichous ascospores. Ascospores are hyaline to straw coloured, fusoid, bicellular with a conspicuous constriction at the septum, finely roughened 12-14.5 x 4.5-6 µm (9.8-13.5 x 4.5-5.2 µm according to Meiffren, 1957). Occasionally 2 or 3 septate ascospores were spotted but the drawings of Booth (1971) suggest a mixture with another species.

Heim & Saccas (1951) reported perithecia production *in vitro*. Booth (1971) also mentioned of perithecia formation *in vitro* by crossing different strains including a "male strain". Nevertheless, meanwhile the "male strain" has been identified as *Fusarium stilboides* and these crossings could not be repeated even by using single ascospore cultures (von Blittersdorff & Kranz, 1976). The latter authors observed hyphal anastomosis between hyphae of these single ascospore cultures. Several authors reported the appearance after 4-6 weeks incubation on malt agar of up to 4 mm thick, branched and cauliflower like sclerotial plectenchyma. After 6 month these structures had not evolve into fertile perithecia. Further studies, based among others on the ecological requirement for ascospore production in nature, should clarify if these failures are due: i) to particular environmental requirement (temperature, humidity, light, substrate, duration, ...), ii) heterothallism requiring the presence in the diseased tree of strains with defined mating type, iii) necessity for "fertilisation" of the

sclerotial plectenchyma. The clarification of this point is important to assess the possibility and extent of gene flow through the sexual cycle and of resulting diversity.

Anamorph formation and diversity

Contrasting with the regular reports of the teleomorph of the wilt fungus on diseased coffee trees, information on the field production of the anamorph is scarce. Steyaert (1948) reported conidia formation on the bark of diseased plants and Fraselle (1950) mentioned formation under high humidity conditions of powdery white patches composed of mycelium producing as well micro- as macroconidia on the necrotic bark, both at the stem base and on the branches of wilted *C. robusta* trees. By incubating surface sterilized, natural infected stem pieces in a humid chamber, Meiffren (1957) observed after 2-3 days abundant production of microconidia and later on macroconidia.

Most of the information concerning the anamorph stage originates from cultures on various nutrient media, starting with Steyaert (1948) who was the first to describe as *Fusarium xylarioides* Steyaert isolates from *C. excelsa* showing symptoms of tracheomycosis originating from Bangui (former Afrique Equatoriale Française).

There appears to be quite a range of variation in the size of the microconidia and macroconidia reported by the various authors. Beside strain-dependent differences, the growing conditions (medium, temperature, light, age) as well as the difficulties for determination of the number of septa may be the cause. Meiffren (1957) and also von Blittersdorff & Kranz (1976) preferred gentian violet in glycerol water to lactophenol cotton blue for septation analysis. By comparing various isolates from *C. arabica* (Ethiopia) with one form *C. excelsa* (Central African Republic) and one from *C. canephora* (Guinea) on various media including malt extract agar (MA), Czapec Dox Agar, potato dextrose agar and beer wort agar, von Blittersdorff & Kranz (1976) concluded that the isolates from *C. arabica* agreed in essential microscopic characteristics such as shape, septation and size of micro- and macroconidia with the isolates from the other coffee species in Central and West Africa. All 3 origins of isolates produced within 3-4 days on MA at 25°C and a 12h photoperiod unicellular curved, allantoid to reniform microconidia. After 5 weeks, the size ranged frequently within 4-14 x 1.5-2.8 (mean 8-11 x 2.1-2.2 µm). The size of macroconidia characterized by a foot cell and a curbed hook ranged for: non-septate conidia within 9-20 x 1.5-2.5 µm (mean 11-13 x 1.9-2.2 µm), one-septate 11-30 x 1.5x3.0 µm (mean 16-21 x 2.3-2.4 µm), two-septate 16-32 x 1.8-3.0 µm (mean 21-25 x 2.6-2.7 µm) and three-septate 20-35 x 2.3-3.4 µm (mean 26-28 x 2.8-2.9 µm). The frequency of microconidia and unicellular

macroconidia, one-septate, two-septate and three-septate macroconidia varied within 21-54, 44-64, 1.4-9 and 0.9-5.0 %, respectively. The difference between micro- and unicellular macroconidia is sometimes tenuous. Meanwhile, SNA became widely adopted as reference medium for characterisation of conidia formation of a range of fungi including *Fusarium* spp. and should also be used for comparison of *G. xylarioides* strains. Considerable variations of growth rate mycelium density and pigmentation were observed according to the isolates and the culture media. These characteristics should be assessed with fresh isolates with no or only limited subcultures on rich media.

Survival structures

Fraselle (1950) observed hyaline chlamydospores in xylem vessels. On culture media scarce production of mycelial chlamydospores was detected for all tested strains (von Blittersdorff & Kranz, 1976). Meiffren (1957) mentioned frequent conidial chlamydospores. Except for the latter case, host tissues need first to be decomposed before these survival structures could be released. On base of the misidentification as *F. oxysporum,* Fraselle (1950) suspected growth and survival of the coffee wilt pathogen in the soil. Jacques-Félix (1954) also considered the pathogen as endemic as a saprophyte in all intertropical African soils under forest or under cultivation. Nevertheless, Meiffren (1957) never succeeded in isolating *F. xylarioides* from soil collected at the base of wilted coffee trees nor from rootlets of wilted trees. The relatively short period of two years for sanitation of the soil between uprooting and destruction of wilted trees and replanting new trees, which has to be found sufficient/which is advised by several authors, suggest a low survival capacity in the soil of *G. xylarioides* compared to *F. oxysporum*. The duration of the sporulation and survival on the stem tissues in nature has not yet been assessed.

Propagation

Most authors consider that ascospore and conidia become dispersed by rain, wind, human traffic and contact between bushes. This is supported by the pattern of disease spread at regional and field level, but specific proof is missing. On the other hand, early detection of diseased trees followed by rapid destruction of the tree on the spot, which restrict propagation of ascospores and conidia, has been found to control wilt progress (Fraselle et al., 1953; Meiffren, 1957; Kalonji, this meeting).

Saccas (1951) mentioned that ascus dehiscence and ascospore projection are favoured by alternate humidity and dryness. However, no data are available at my knowledge

concerning the seasonal variation in ascospore or conidia formation and dispersal. A method for monitoring of perithecia and conidia production on naturally infected coffee trees is proposed in annex. Observations *in vivo* should also clarify if conidia are produced in mucilage requiring rain splash dispersal or dry allowing easy wind dispersal.

The possible role of insects, birds and other animals in dispersal of spore or contaminated soil and residues should also been kept in mind.

Most of the published work is related to propagation to neighbouring plant within a plantation creating conspicuous disease foci. Long distance propagation causes generally first disease on isolated trees. Establishment of *G. xylarioides* on the wild *Coffea* spp. or other trees in the forest, could represent a serious treat because of a source of inoculum to bordering plantations, even if eradication measures are taken there. Wilting of the susceptible native genotype could also cause a decrease in coffee biodiversity.

The existence of alternate hosts to *G. xylarioides* has not yet been investigated systematically. *F. xylarioides* has been reported from other plants such as rye rhizosphere (Kurek & Jaroszuk, 1994). Such statements needs to be confirmed and the pathogenicity on coffee of the isolates from other hosts tested.

As with other vascular wilts mycelium colonisation could theoretically extend to the berries and the seeds. We are not aware of any direct proof of this hypothesis but spread of coffee wilt in Uganda has been associated with the move of coffee berries for processing to distant areas. Pochet (1988) discouraged harvest of berries from diseased trees.

Infection

From numerous observations of wilted coffee trees, Fraselle et al. (1953) and Meiffren (1957) concluded that infection occurs through wounds on the stem base or on big roots close to the surface. Infection was never detected on upper parts of the stem, even damaged by stem borer, without being also isolated at the stem base. Nevertheless, the questions raised already by Meiffren (1957): "above or below the soil surface?» and "are there predisposing factors for infection", have unfortunately not yet been addressed. The answers to the questions: "By what? Where? When? Why infection occurs? are important for the design of rapid greenhouse screening methods for field resistant genotypes, as well as for recommendations of crop management practices.

Colonisation

Most of the vegetative growth phase of *G. xylarioides* occurs in the xylem. Fraselle (1950) reported abundant colonisation of the large vessels of young wood by hyaline, septate, 3.5 to 5.2 µm in diameter, loosely clustered hyphae. The mycelium extending later to the fibres surrounding the vessels appeared thinner. Colonisation to the centre of the stem as well as to the bark occurs through the medullar rays. Thylosis and gummosis are also observed. The wilt symptoms are associated with impediment of water transport through the above alterations and mycelium plugs. Meiffren (1957) also suspected involvement of toxins because of wilt induction by cell-free culture filtrate. The black purple colour of the xylem results rather from host tissue alteration/reaction than from hyphal pigmentation.

In the bark, colonisation appeared less conspicuous except the perithecia bearing plectenchyma developing in the cracks of dead bark tissues. This suggests that xylem colonisation proceeds bark colonisation at least in the upper part of the stem. Nevertheless, some authors mentioned thickening of the bark at the stem base due to a phloem hyperplasia, as a possible mechanism of resistance to colonisation. This would rather suggest a growth from the bark to the xylem. Bouriquet (1959) associated the resistance of Robusta with a higher chlorogenic acid content in the wood compared to Kouilu; 16mg/100g fresh material compared to 4mg/100g.

Incubation and latency periods

Because of the uncertainties concerning the infection, other important parameters in the life cycle of the coffee wilt pathogen such as the duration of the incubation and latency periods, are unfortunately also still poorly documented. Saccas (1951) reported that incubation period varies from 6 to 10 days for 2- to 3-month old trees to 4 to 6 months for 7- to 10-year old plants. Nevertheless, in 1956, Saccas stated that for trees over ten years of age the incubation period is usually more than eight months and frequently more than one year. The incubation period probably varies according to climate, genotype, plant age, and pathogen aggressiveness... This is also a key issued for epidemiological studies and control measures.

Mycological and pathogenic diversity

How is the life cycle of *G. xylarioides* related to mycological and pathogenic diversity? Spontaneous mutations or genetic reorganisation through a parasexual and a sexual cycle may create diversity within *G. xylarioides*. In Ubangui, Saccas (1953) distinguished 3

races on base of morphological and pathogenic criteria. In Ivory Coast, Meiffren (1957) found no host specialization for strains isolated from *C. canephora* or *C. abeokutae* but two types based on colony and conidial morphology. Central and West Africa isolates from *C. excelsa* and *C. canephora* can be distinguish from East African *C. arabica* isolates at IGS level (Brayford & Flood, this meeting), but they share the same basic morphological characteristics (von Blittersdorff & Kranz, 1976). Nevertheless, *C. arabica* appears to be resistant to the *G. xylarioides* causing wilt on the other *Coffea* spp. suggesting diversity at pathogenic level.

We plan to perform at UCL inoculations under controlled conditions combined with diversity studies at molecular level in order to clarify the possible existence of formae speciales such as f.sp. *canephora* and f.sp. *arabica* within *G. xylarioides*. Progeny analysis of single asci as well as analysis of the stability or exchange of genetic markers after pairing in vegetative compatible groups and reproduction of the sexual cycle may help understanding the genetic structure of the *G. xylarioides* populations. It will also allow assessment of the possibility of gene flow and of the risk of occurrence of new genetic combinations able to bypass cultivar resistance.

Saprophytic or pathogenic "relatives" of *G. xylarioides* have not yet been identified. Phylogenetic studies of *Gibberella* spp. especially from Central and Eastern Africa where coffee wilt has been reported, such as *G. stilboides* causing the "Storey bark disease" may shed some light on this point. The link with *Fusarium oxysporum* f.sp. *coffeae* causing coffee wilt in Parana (Cardoso, 1986) will also be explored.

References

BOOTH, C. & WATERSTON, J.M. 1964. C.M.I. Description of Pathogenic Fungi and Bacteria. N° 24, C.M.I., Kew, England.

BOOTH, C. 1971. The Genus Fusarium. CMI, Kew, England, pp.115-118.

BOURIQUET, G. 1959. Plant diseases and pests in some African territories. FAO Pl. Prot. Bull., 7: 61-63.

CARDOSO, R.M.L. 1986. Ocorrência da murcha vascular do cafeeiro (*Coffea arabica*) no Estado do Paranà-Brasil, induzida por *Fusarium oxysporum* f.sp. *coffeae*. Fitopatologia Brasileira, 11: 753-760.

FRASELLE, J. 1950. Observations préliminaires sur une trachéomycose de *Coffea robusta*. Bull. Agricole du Congo Belge, 41: 361-372.

FRASELLE, J.C., VALLAEYS, G., DE KNOP, O. 1953. La lutte contre la trachéomycose du caféier à Yangambi et le problème que pose actuellement cette maladie au Congo Belge. Bulletin d'Information de l'INEAC, 2: 373-394.

HEIM, R. & SACCAS, A.M. 1951. La trachéomycose des *Coffea excelsa* et *robusta* des plantations de l'Oubangui – Chari. C. rend. Acad. des Sc.,11 septembre 1950, 231: 536.

JACQUES-FELIX, H. 1954. La carbuncullariose. Bull. sci. Minist. Colon. Sect. Agric. Trop. 5 (Contributions à l'étude du caféier en Côte d'Ivoire), pp.296-344.

KRANZ, J. & MOGK, M. 1973. *Gibberella xylarioides* Heim et Saccas on Arabica coffee in Ethiopia. Phytopath. Z., 78: 365-366.

KUREK, E.& JAROSZUK, J. 1994. Occurrence of beneficial and deleterious for plant growth *Fusarium* strains in rye (*Secale cereale* L.) rhizosphere. Acta Microbiologica Polonica, 43: 181-187.

MEIFFREN, M. 1957. La trachéomycose. Dans: Les maladies du caféier en Côte d'Ivoire. Haut Commisariat de l'A.O.F. Centre de Recherches Agronomiques de Bingerville. Côte d'Ivoire, pp. 9-54.

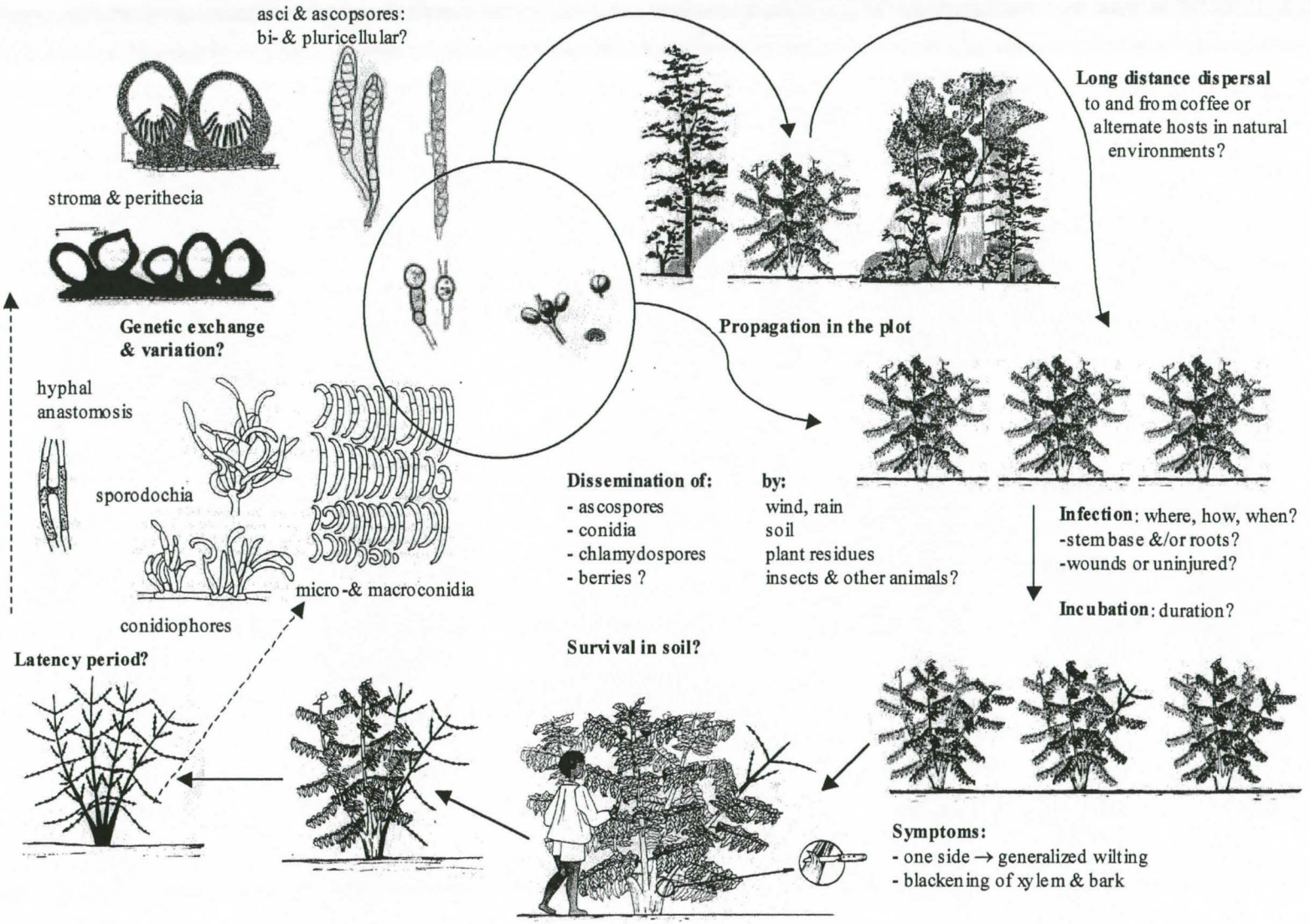
SACCAS, A.M. 1951. La trachéomycose (carbuncullariose) des *Coffea excelsa, neo-arnoldiana* et *robusta* en Oubangui-chari. Agron. trop. 6 : 453-506.

SACCAS, A.M. 1953. Les variations dans l'aspect macroscopique et biométrique du *Fusarium xylarioides* Stey., agent de la trachéomycose des *Coffea*, liées aux races physiologiques. Atti del VI Con. Intern. Di microb., Roma.

SACCAS, A.M. 1956. Recherches expérimentales sur la trachéomycose des caféiers en Ubangui-Chari. Agron. trop., 11 : 7-38.

STEYAERT, R.L. 1948. Contribution à l'étude des parasites végétaux du Congo Belge. *Fusarium xylarioides* n.sp. Bull. Soc. Roy. Bot. de Belgique, 80, 2ème série 30: 42-48.

VON BLITTERSDORFF, R. & KRANZ, J. 1976. Vergleichende Untersuchungen an *Fusarium xylarioides* Steyaert (*Gibberella xylarioides* Heim et Saccas), dem Erreger der Tracheomycose der Kaffees. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 83: 529-544.



The life cyle of the coffee wilt pathogen *Gibberella xylarioides*, anamorph *Fusarium xylariodes*

H. Maraite, UCL *Drawings from Pochet, 1988; von Blittersdorff & Kranz, 1976; Meiffren, 1957; Booth, 1971; Van den Abeele & Vandenput, 1956.*

Annex 2
COWIDI – ICA4 – 2000 – 10312

Proposal of a method for

Monitoring of perithecia and conidia production of *Gibberella xylarioides* on naturally infected coffee trees

Henri Maraite, UCL

Contribution to

WP 1 : Pathogen diversity
Task 4 : Description of the fungal life cycle, asexual and sexual phases (P1, P2, P3, P4)

WP4 : The disease epidemiology
Task 3: Define the conditions conducive to the appearance of the sexual phase and its importance in the spread of the disease (assessment of ascospore production in the field)(P2, P3, P4)

Choice of the location(s)

Easy of access, coffee plants at various wilting stages, close to a weather station recording rainfall, temperature, relative humidity (sunshine duration) at a daily or hourly base.

Example: Coffee Research Centre , Kituza

Identification of coffee trees for sampling

In one or several plots, if possible of known clones, identification of 3 trees showing:

- first symptoms (leaf wilting/dropping on one stem tip, less than 1/3 from top),
- symptoms on more than half of stem height, or
- the whole stem(s) wilted.

By carefully scratching away the bark on limited spots (max. 1 cm ∅) at the base of the stem (2-3 cm above the soil layer), at 27 cm, 57 cm and 87 cm height, the extent of the blackened wood area is delimited (Fig. 1). A sampling grid drawn or printed on a transparency film (Fig. 2) is centred on the blackened area, upper line at 20 cm above soil layer. Stainless steel brads are nailed laterally on 3 points for easy repositioning of the grid during successive samplings. Similar sampling areas are defined with the upper grid line at 50 and 80 cm (Fig. 1).

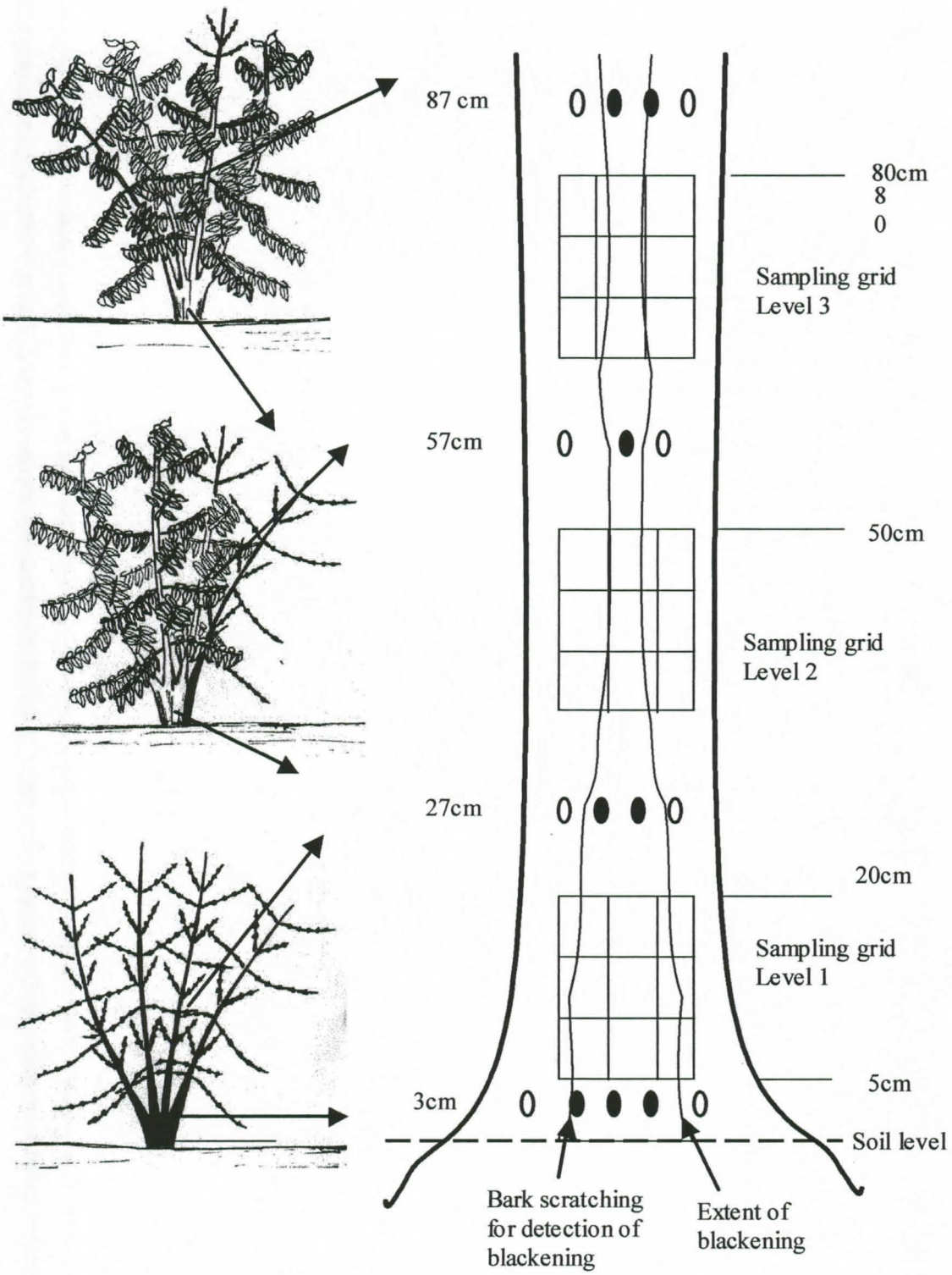


Fig. 1. Type of diseased coffee trees selected for monitoring during one year *G. xylarioides* sporulation and outline of the observations for delimiting the infected bark area and positioning the sampling grid.

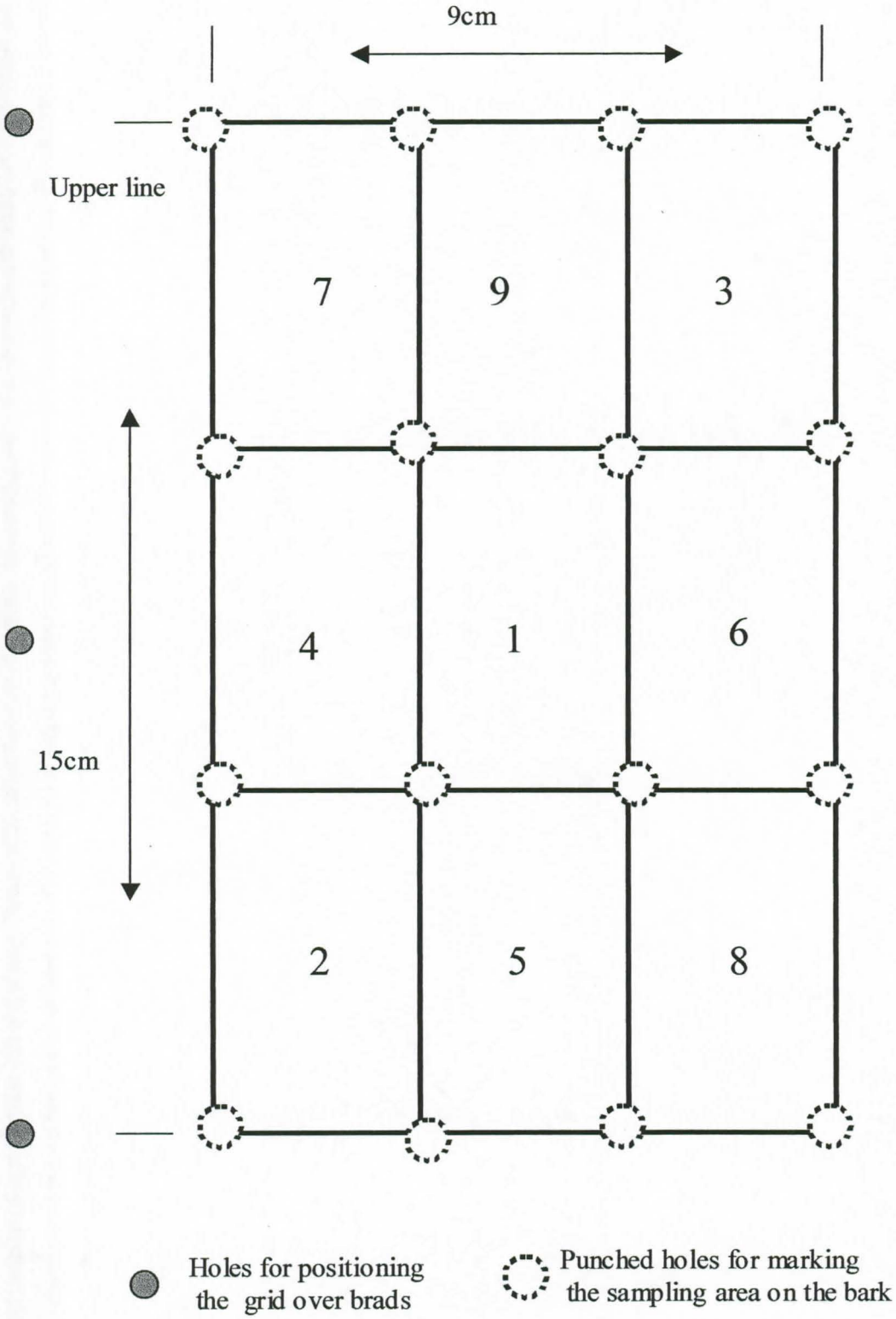


Fig. 2. Sampling grid with a proposed sequence of sampling

The trees are numbered and the health status described. The presence of injuries through which infection my have occurred and the extent of bark blackening are noted. A layout showing the north and the position of the sampled trees in the plot as well as the side of sampling is prepared.

Sampling

Seen the lack of knowledge concerning the period of conidia formation and ascospore release a systematic sampling every 6 weeks is proposed, starting as soon as possible.

On a sampling date the grid is positioned through the nails and the corners of the area to be sampled marked with a waterproof felt pen. With help of a knife a 1,5 to 2 cm x 4,5-5 cm piece of bark is cut between the marks and transferred to sampling boxes (f.i. Cardboard Micro slide folders, type Thomas scientific 6708M10) lined with double face adhesive tape.

The health status of the stem and the tree is assessed and the appearance of the sampling areas noted (humidity, visible mycelium, conidia production, perithecia).

If during the sampling, areas of interest are spotted outside the area sampled or on other stems, bark pieces are also taken for analysis in the laboratory.

Analysis in the laboratory

The sampling boxes are kept at room temperature under dry condition.

Under the stereomicroscope 3 areas of 10x10 mm are analysed for each sample and the abundance of perithecia rated:

0= no perithecia

1= up to 3 perithecia single or in groups /cm^2

2= up to 10

3= more than 10 perithecia/cm^2

The status of the perithecia could be evaluated as :

S dark stromata without perithecia

G stromata with ostiolate perithecia

E empty degraded perithecia

Individual perithecia are taken with a scalpel fitted with a n° 11 blade crushed and mounted in a drop of lactophenol cotton blue for verification with the *G. xylarioides* description and for examination of asci and ascospores maturation.

The same procedure applies for identification of the anamorph stage.

In case of detection of mature perithecia the samples are forwarded to U.C.L. for trials to isolate the 8 ascospores of same asci.

These single ascospore stains will allow study of the genetic base of pathogen diversity.

NATIONAL AGRICULTURAL RESEARCH ORGANISATION (NARO)

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

First Annual Report (November 2001 to October 2002)

Coffee Research Institute (CORI), Kituza
P.O. BOX 185
Mukono
UGANDA

Work Plan 1: Studies on diversity of *F. xylarioides*

Dr. G.J. Hakiza

Materials and Methods

Sample collection

Plant materials showing typical symptoms of coffee wilt disease were collected from farmers' fields in the districts shown in Table 1. Infected branches and stems were arbitrarily selected from sampled trees and cut off with secateurs into pieces of 15 – 20 cm long. These were wrapped in newspapers or envelopes and transported to the laboratory at ambient temperatures. Isolation of the pathogen was done with 1 – 7 days from collection. In addition, collections were made from 10 robusta genotypes from the robusta hybrid trial at Kituza and from *Coffea excelsa* in the coffee collection at Kawanda Agricultural Research Institute.

The following are the clones from which *F.xylarioide* isolates were obtained

1.	L/2/7	63.4%	Wilt incidence under field conditions
2.	P/3/6	71.3%	"
3.	B/1/1	29.0%	"
4.	223/32	20.0%	"
5.	$1^{s}/3$	12.5%	"
6.	257/53	37.5%	"
7.	C/6/1	17.0%	"
8.	Q/1/1	50.0%	"
9.	G/3/7	38.0%	"
10.	H/4/1	69.0%	"

(Source: Annual Report 2000-2001 CORI)

Primary isolation

In the laboratory the stems, which varied in diameter from a few mm to 6 cm, were split in halves, lengthwise. Suitable pieces of about 20 mm were trimmed off from the margins of the infected tissue and surface sterilized in 2 % Jik for 3 minutes, followed by rinsing in three rounds of sterile distilled water. Excess moisture was removed with sterile tissue paper and aseptically transferred onto Petri dishes (9cm) containing 2% Tap Water Agar (TWA) and incubated at ambient room temperature (23 – 25 C) with a 12 hr photoperiod using fluorescent lights. Three to four days later, the dishes were examined and any growth was identified.

Single Spore Isolates

From the cultures, single spore isolates were made as follows. Thin agar plates of TWA were made in Petri dishes. To make it easier to observe germinating spores, a line was drawn on the reverse of the Petri dish with TWA using a marking pen.

Three additional lines, about 20 mm apart were drawn at right angles to the first line. A thin spore suspension of each isolate was made and with the use of a sterile loop, the suspension was streaked on the TWA along the lines drawn with the marking pen. This was done last thing in the evening and the dishes were incubated over night under conditions stated earlier. The next day the plates were examined under low power magnification (x20) of the microscope, and well-separated germinating spores were carefully cut out and aseptically transferred onto required media and incubated as before. Cultures were maintained for future use on slants of Synthetic Nutrient Agar (SNA) slants in 10 ml bottles with screw tops.

Table 1. Isolate code number and geographic origin of the 15 F. xylarioides isolates from Uganda being used for diversity studies

Isolate code	S/county	County	District
F1	Kakindu	Busuzu	Mubende
F2	Kasambya	Buwekula	Mubende
H1	Kasawo	Kasawo	Mukono
L	Kibibi	Butambala	Mpigi
M	Kebisoni	Rubabo	Rukungiri
O	Busana	Busana	Kayunga
P	Wakiso	Wakiso	Wakiso
Q	Bukulula	Kalungu	Masaka
R	Butiti	Mwenge	Kyenjojo
S	Masaka	Bugangaizi	Kibale
T	Kalagala	Bamunanika	Luwero
U	Ntwetwe	-	Kiboga
V	-	-	Kalangala
W	Muwanga	Kiboga	Kiboga
X	Bubandi	Bwamba	Bundibugyo

Pathogenicity tests of isolates to selected robusta clones

Robusta clones are being raised in the nursery, of the 6 clones recommended for planting in Uganda. These are not resistant to CWD. The breeder will provide other clones for use as differential plants. Inoculations will be carried out in 3 months' time when the clones are 5 - 6 months old.

The protocol developped is:

- 10 -12 plants / clone for each of 4 replicates
- Clones / cuttings of 6 - 8 months were used in root dip, stem inoculation
- Spore density 1.3 x 10^6, conidia / ml, made from mixtures of isolates from Kituza, Mubende and Mpigi districts.

The records collected are the incubation period, incidence of CWD 3 days from symptom development for 90 days and days to death of plants from symptoms.

Plants are considered dead when completely wilted and defoliated. ANOVA is carried out for significant variations in reactions among clones.

Comparison of inoculation methods

Robusta clones for comparison of inoculations by root dipping and stem injections are being raised. They will be ready for inoculations in November 2002.

WORK PLAN 3: BREEDING FOR RESISTANCE

Pascal Musoli

I INTRODUCTION

Coffee wilt disease (CWD) was first reported in Uganda in 1993 in one out of 44 districts growing coffee that borders the Democratic Republic of Congo. To date CWD has spread to all 30 districts traditionally known for growing robusta coffee and it is destroying the coffee at an alarming rate. In some districts such as Kibale, Hoima, Mubende, Mukono and Kayunga, more than 50% of the farms have been destroyed and in some farms the destruction is 100%. CWD is now of a major concern in Uganda and yet the recommended control methods basing on cultural management practices such as uprooting and burning are not effective, difficult to implement and costly. Breeding for resistance is being considered as the most likely cost effective method in the long run. This work plan 3 under this project was designed to initiate studies that can lead to breeding varieties with durable resistance.

II OBJECTIVES

The objectives of WK3 are to:

1) Identify sources of resistance to CWD through screenhouse tests on young seedlings and cuttings and field assessments
2) Assess the inheritance of resistance to CWD
3) Define a breeding strategy towards durable resistance.

III METHODOLOGY

The work is structured under 6 tasks viz:

1 Identification of sources of resistance

The search for resistance involves assessment of germplasm locally available in Uganda and from exotic sources, mainly other African countries with history of having controlled CWD using resistant varieties. The local germplasm include robusta and the robusta intraspecific hybrids, arabica and the arabica intraspecific hybrids, arabusta (interspecific hybrids between robusta and arabica) and other coffee species available in the germplasm collections/fields at CORI/KARI. Local germplasm also include the coffee trees surviving in wilt 'hot spots'.

2 Collect, bedding/dispatching seed and cuttings

Seeds and cuttings are to be harvested from all robusta and arabica genotypes, in germplasm plots at CORI and CWD 'hot spots', for raising into seedlings and rooted cuttings in coffee nurseries. Seedlings and cuttings are also to be harvested from the intraspecific hybrids of robusta and arabica and selected interspecific arabusta hybrids. The seeds and cuttings from the hybrids will also be raised into seedlings

and rooted cuttings in the coffee nurseries. The seeds and cuttings will be raised in the nursery at CORI and part of the seed lots of some of the genotypes will be dispatched to CIRAD for similar studies.

3 Conduct screening tests on seedlings and cuttings

Seedlings and cuttings of all genotypes being assessed will be inoculated using protocols developed in partnership with pathologist. The inoculated plants will be scored for response to CWD infection. Wilt resistant genotypes will be selected and planted in multiplication plots at CORI.

4 Multi-locational field trials with tolerant/resistant varieties

Cuttings will be harvested from the CWD resistant/tolerant lines in the multiplication gardens and rooted in a nursery at CORI. The rooted cuttings will be planted in on-farm and on-station field trials in CWD 'hot spots' in different coffee growing locations. The on-station trials will be at CORI. The lines planted in multi-locational trials will be assessed for response to CWD under field conditions under different farmers' management and different environments. They will also be assessed for yield, quality and resistance to other major coffee diseases such as red blister and leaf rust.

5 Analysis of heritability of the resistance

Crosses will be made between CWD resistant/tolerant lines in the field trials at CORI with wilt susceptible lines using different crossing models such as the half sib, NC2 and diallels. Crosses will also be made among the resistant/tolerant lines. All the hybrid progenies generated will be inoculated and scores of their responses will be used in determining the heritability of resistance/susceptibility. Assess will also use responses of open pollinated F2 hybrids of selected arabusta. The assessment of seedling of open pollinated seeds of different genotypes (resistant/tolerant and susceptible), as half sibs, will also be used to compute heritability.

6 Proposal for a breeding strategy towards durable resistance

Basing on studies on the screening responses and heritability, a strategy for breeding durably resistant varieties will be proposed.

IV) RESULTS/PROGRESS

1 Identification of sources of resistance

Following a long period without research funds, the germplasm plots on-station at CORI were very weedy and therefore the initial activity was to rehabilitate them so as to induce the coffee into vegetative and reproductive productivity, for generating cuttings and seed/seedlings respectively, and therefore provide the materials for studies. All the germplasm collections were rehabilitated through controlling weeds (slashing, herbicide application), stumping and desuckering.

Consequently, the genotypes, especially of robusta coffee including the intraspecific hybrids, were induced into vegetative and reproductive production. Arabica was rehabilitated later and are only recovering from effect of the weeds.

During a baseline survey on CWD in Uganda, conducted between March and May 2002, it was established that in the districts of Mukono, Bundibugyo, Rukungiri, Kayunga, Kibale, Hoima, Kiboga, Mubende, Luwero, Wakiso and Mpigi, there are coffee farms which, are nearly 100% devastated but with a few scattered survivor genotypes. These farms are therefore potential sources of additional materials for screening.

A variety trial field at Kituza exposed to CWD has exhibited differences in variety responses to the diseases. Some of the lines in this trial have remained unaffected by the disease and they could be resistant to CWD and therefore are ideal for inclusion in the screen house assessment.

Ivory Coast has been identified as the possible source of exotic germplasm and a memorandum of understanding between the two countries is being discussed.

2 Collect, bedding/dispatching seed and cuttings

Harvesting and rooting of cuttings at CORI coffee nursery has started. So far genotypes rooted are indicated in tables 1-3. Harvesting seeds of most of these genotypes will be during November 2002-January 2003 harvest season.

Table 1:Seedlings and cuttings of robusta coffee genotypes
under nursery at CORI. Harvesting/planting date May 15 to Aug. 29, 2002
Expected date due for inoculation November 2002-February 2003

No.	Variety	Cuttings	Seedlings	No.	Variety	Cuttings	Seedlings
1	1s/2`	574	1200	33	'3/71	100	
2	1s/3	325	1100	34	'4/6	274	
3	1s/6	312		35	'14/1	20	
4	3s/6	62		36	'14/19	16	
5	'1/2	23		37	'14/50	38	
6	'1/3	10		38	'14/58	21	
7	'1/4	4		39	'14/60	61	
8	'1/5	17		40	'14/61	27	
9	'1/11	54		41	'14/70	43	
10	'1/12	118		42	'47/66	44	
11	'1/15	213		43	'202/21	58	
12	'1/17	17		44	'202/33	58	
13	'1/48	25		45	'202/36	20	

No.	Variety	Cuttings	Seedlings	No.	Variety	Cuttings	Seedlings
14	'1/70	33		46	'202/62	46	
15	'1/71	26		47	'202/63	201	
16	'2/7	49		48	'203/14	28	
17	'2/11	20		49	'203/30	300	
18	'2/13	34		50	'203/32	261	
19	'2/57	17		51	'203/66	75	
20	'2/67	13		52	'203/72	45	
21	'3/5	31		53	'203/74	104	
22	'3/9	26		54	'209/29	135	
23	'3/12	27		56	'209/39	26	
24	'3/14	18		57	'213/35	35	
25	'3/15	43		58	'213/64	44	
26	'3/20	50		59	'214/70	90	
27	'3/54	26		60	'218/32	177	
28	'3/56	46		61	'286	264	
29	'3/59	25		62	'288	129	
30	'3/62	91		63	'222/65	165	
31	'3/63	45		64	'223/32	130	
32	'3/66	12		65	'223/38	40	
66	'223/76	39		101	'257S/2	50	
67	'223/79	98		102	'257s/8	69	
68	'224/25	40		103	'257/18	67	
69	'224/64	51		104	'258/1	73	
70	'225/65	36		105	'258s/58	378	
71	'226s/11	221		106	'258s/58/3	61	
72	'227/2	56		107	'258/63	92	
73	'227/19	35		108	'258s/70	106	
74	'227/54	45		109	'260s/22	41	
75	'227/56	50		110	'261s/2	477	
76	'227/58	200		111	'261s/15	13	
77	'227/61	206		112	'261s/21	228	
78	'228/13	50		113	'266s/11	160	
79	'228/15	38		114	'267s/3	36	
80	'228/57	130		115	'267s/6	250	
81	'228/65	23		116	'267s/15	155	
82	'229/58	11		117	'267/15	27	
83	'234/37	304		118	'268/267/24	54	
84	'238/29	300		119	'268/267/25	69	
85	'245/21	99		120	NGREDOG/3	44	
86	'245/25	165		121	J1/14/1	10	
87	'245/62	209		122	J1/14/19	17	

No.	Variety	Cuttings	Seedlings	No.	Variety	Cuttings	Seedlings
88	'247/30	48		123	J1/14/21	10	
89	'247/61	93		124	J1/14/48	3	
90	'247/62	42		125	J1/14/50	13	
91	'247/69	138		126	J1/14/51	36	
92	'247/71	14		127	J24/13/5	92	
93	'254/28	683		128	J24/13/12	36	
94	'254/37	37		129	J24/13/22	17	
95	'254/64	27		130	J24/13/52	48	
96	'254/70	20		131	J24/13/53	72	
97	'254/74	167		132	J24/13/59	60	
98	'254/76	20		133	J56/20/5	10	
99	'254/79	205		134	J56/20/57	36	
100	'254/80	478		135	J56/20/60	26	
136	J56/20/61	19		150	G/3/7	100	
137	J94/2/13	19		151	H/4/1	24	1000
138	J105 203/11	48		152	J/1/1	42	1200
139	J105 03/1	70		153	L/2/7	46	
140	J124.9/1	24		154	P/3/6	29	1150
141	JB5109.4/2	48		155	P/5/1	21	
142	JB5109.4/3	25		156	Q/1/1	51	
143	JB5109.4/5	48		157	Q/3/4	75	1200
144	B/1/1	65		158			
145	B/2/1	26		159	Q/6/1	38	
146	B/6/2	34		160	R/1/4	70	
147	C/6/1	58		161	'257S/53	18	
148	C/1/7	28		162	'258S/24 (O)	48	
149	E/3/2	25		163	'223/32	55	

Table 2: Cuttings and seedlings of selected F1 arabusta interspecific hybrids under nursery at CORI

	Variety	Cuttings	Seedlings	Harvest/planting date	Expected date due for inoculation
1	3/71 CS 20 X (35/2`X134/4-62)/10	0	340	25/7/2002	January 2003
2	3/71 CS 20 X H148/11	25	11	"	"
3	3/71 CS 20 X H148/15	230		"	"
4	245/25 CS 20 X H148/3	70		"	"
5	245/25 CS 20 X H148/9	20		"	"
6	245/25 CS 20 X H148/10	326			"
7	245/25 CS 21 X H148/7	187		"	"
8	236/26 CS 14 X RUME 14/12	116		"	"
9	236/26 CS 13-69 X SL14/28/2/9	15		"	"
10	236/26 CS 9-69 X SL14/28/2/2	7		"	"

More cuttings will be harvested as materials become available. Dispatch of materials to CIRAD awaits signing of Material Transfer Agreement between CORI and CIRAD.

3 Conduct screening tests on seedlings and cuttings

All the materials being raised will be assessed in the screenhouse at Kituza (Uganda) and CIRAD (France) for response to CWD. This however requires a standard inoculation protocol to be able to relate finding at CIRAD and Kituza. The protocol is to be worked with pathologist starting October/November 2002. But prior to this project, a limited number (10-12) a number of arabica and robusta seedlings were tested by dipping their roots into a suspension of mixed field isolates of *Fusarium xylariodes* at a concentration of 1.3×10^6 spores per ml. Through this approach cuttings of arabusta were also inoculated and seedlings and cuttings from 7 wilt devastated farms were also assessed.

20 clonal varieties planted in a variety comparative trial at CORI were assessed for their response to CWD under field conditions. Results of this trial starting January 1999 to June 2002 are shown in table 4.

Table 3: CWD incidence (1999-June 2002) and yield (2001/2002) of 20 robusta coffee varieties in a comparative trial at Kituza

	Variety	Mean infected & dead plants	% incidence	Mean yield (01/02)
1	P/5/1	5.5a	45.8	-
2	H/4/1	5ab	41.7	-
3	P/3/6	5ab	41.7	646.6bcd
4	B/6/2	5ab	41.7	-
5	C/1/7	4abc	33.3	194.2def
6	257/53	4abc	33.3	53.25f
7	E/3/2	4abc	33.3	-
8	Q/1/1	3.5bc	29.2	392.5def
9	Q/6/1	3cd	29.2	640.7bcd
10	B/2/1	3cd	29.2	98.8ef
11	223/32	3cd	29.2	195.0def
12	G/3/7	3cd	29.2	125.0ef
13	L/2/7	1.5de	12.5	126.1ef
14	B/1/1	1.5de	12.5	467.0cdef
15	$1^S/2$	0.5e	4.2	589.9bcde
16	C/6/1	0.5e	4.2	1436a
17	$1^S/3$	0.5e	4.2	900.9bc
18	Q/3/4	0.5e	4.2	446.6cdef
19	R/1/4	0.5e	4.2	651.5bcd
20	J/1/1	0e	0	999.9ab
	LSD	**1.938**		**447.6**
	C.V	**34.6%**		**42%**

Variety J/1/1 has not shown any CWD wilt symptoms. The variety will be challenged with CWD inoculum under screenhouse to validate the field observations. It has also been included in field trials planted in Mukono (Kituza), Mubende and Luwero to validate its response under different agro-ecological conditions.

4 Multi-locational field trials with tolerant/resistant varieties

This task awaits the screen house tests. However during the small tests conducted prior to this project, a 372 genotypes belonging to different varieties of robusta survived two rounds of challenge with a mixture of *Fusarium xylarioides* isolates. 25 arabusta clones and seedlings of 7 arabica varieties also survived. All the robusta survivors were planted in a mother garden for vegetative propagation. 106 of the genotypes have started producing suckers/vegetative propagules and cuttings have been harvested and are being raised in the nursery shade at CORI. The first lot of cuttings raised were planted in field trials at Kituza (CORI) in April and September 2002. 10 selected arabusta were also planted in clonal comparative trial in wilt infected gardens at CORI, Mubende and Luwero districts.

5 Analysis of heritability of the resistance

Crosses to for this analysis have been initiated. These crosses are still very limited but more will be made in December 2002-February 2003 flowering period. The analysis also awaits assessment of half-sib progenies of robusta genotypes from the germplasm collections at CORI and collections from CWD hot spots and F2 progenies of arabusta hybrids..

6 Proposal for a breeding strategy towards durable resistance

Will be after obtaining results of the other tasks.

V CONSTRAINTS

The major factors that deterred progress during the reporting period include:

i) Lack of Material transfer agreement has prevented dispatch of the available coffee materials (cuttings to CIRAD) where part of the studies will be conducted, especially involving testing the resistance to isolates of *Fusarium xylarioides* from other countries like Cameroon, Ivory Coast, Democratic Republic of Congo and Ethiopia.

ii) Absence of a standard inoculation protocol agreeable to CIRAD and CORI has also affected the mass inoculations.

iii) Limited number of wilt resistant clones has hindered progress in pollination.

iv) Although the project contracted indicates the project was to start in November 2001, the funds were credited to CORI account in January 2002. Further delay was due to paper work at the Bank in Uganda. Consequently the funds became available for work in late February.

v) Although CORI's budget for first year of the project was 75,592 Euro, only 40,000 Euro was remitted to the account. These funds were not sufficient to cover the capital equipment (vehicle and computer) and the recurrent cost of rehabilitation and maintenance of germplasm gardens, field visits to collect plant materials from wilt hot spots.

Work Plan 4: Epidemiology of Coffee Wilt Disease (CWD)

Dr. G.J. Hakiza

Selection of field sites for epidemiological observations on CWD

Four sites for the above trial have been selected in Iganga and Mayuge districts of Eastern Uganda where disease pressure is still low. Hopefully this will allow us to take data over a longer period of time. For comparison, four other sites were also selected in Masaka and Rakai. Disease recording is planned at 4 weekly intervals.

- Selection of field sites
  All are smallholder farms, mostly intercropped with bananas and varied in size from 0.5 ha – 3 ha. All are seedling coffee.

  Selected 8 rows x 16 rows = 128 trees for observation over time. Infected trees, healthy at time of selection were assessed.

UNIKIN

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

First Annual Report (November 2001 to October 2002)

University de Kinshasa
B.P. 866, Kinshasa XI RDC

WP 1. PATHOGEN DIVERSITY

1.1 Sample collection, identification, storage and dispatch of isolates

Attempts were made to isolate *F. xylarioides* from samples (pieces of stems) taken on sick trees in 1995-1998in North East (Isiro, Province Orientale,) and in the East (Beni-Butembo, North Kivu). They were unsuccessful. These samples had been kept in ambient conditions in order to evaluate the duration of survival of the fungus.

Out of the 27 isolates maintained on gelose medium two were identified as *F. xylarioides*, namely isolate SAM from Beni (North Kivu) and isolate I.17 from Isiro (Oriental Province).

These isolates are being maintained on nutritive gelose (Agar?) medium by successive transplantations. They will be used for screening and dispatched to the partners. Aggressiveness has not yet been tested.

The planned epidemiological surveys in Northern and North Eastern DRC will allow the collection of new samples that will enrich the isolates collection. Indeed the political situation that prevented us from any visit has ended, and surveys are planned for December 2002.

WP2 HOST PATHOGEN INTERACTION

With the purpose of screening plant materials a collection is being made from plants identified in Kiyaka Research Station. The general phytosanitary state of this Station and the date it was visited did not allow collecting large quantities of seeds. However most of the seeds collected in 2002 were dispatched to Cirad (France) as per Table 1. KR Nos. are individual plants earmarked within fields planted with a mixture of the 7 selected clones (see below) that cannot be easily identified (KR stands for Kiyaka Robusta).

Table 1: List of seed samples dispatched to Cirad in 2002

No.	Mother Genotype	No. of seeds	Weight (g)
1	KR A/1	222	55
2	KR A/4	11	3
3	KR B/11	824	209
4	KR B/12	65	18
5	KR B/14	624	158
6	KR 1/3	693	122
7	KR 2/5	2078	376
8	KR 9/8	380	100
9	KR 12/4	585	146
10	KR 16/5	424	90
11	KR 19/12	873	161

In the future we should be able to visit the fields at the right stage to collect enough seeds, and to diversify the genetic and geographic sources. Robusta will be collected at Yangambi Research station, and Kouillou at Luki.

New inoculations as planned will be undertaken as soon as we have collected new isolates in the affected areas.

It is planned to inoculate at 4, 6, and 8 months depending on wood formation in the seedlings.

WP3 BREEDING FOR RESISTANCE

Assessments on the reaction of various planting materials to the disease will be made at Yangambi, a Station of INERA (Institut National pour l'Etude et la recherche Agronomique).
This station lays along river Congo, at 0°49' N, 24°29' E, at 470 m a.s.l. The climate is continental equatorial without clear dry season (Af type in Köppen's classification). Rainfall can reach 2,000 mm, average minimum temperature is 19.5 °C, and average maximum temperature is 30°C, humidity is close to saturation.
CWD was observed for the first time in 1939 at Yangambi on plant material originating from Bangui (Central Africa). Later on in 1950 attacks reaching 30-40% dead trees were mentioned in some of the coffee fields.
Assessments have been made since 1977; they are reported in Tables 2, 3, and 4. More detailed results will be reported after we visit the station. Also during the visit to the station and to other sites classes of reaction to the disease will be defined.

3.1 Field "Parc à bois", Yangambi

This field is part of Yangambi collection. It was established in 1964 with cuttings of the recommended clones (5 clones). It is made of 6 blocks of 10 plants per clone.

Results in Table 2 indicate that clones L251, L215, and L147 are the most susceptible, followed by clone SA158. This is a pity, because L251 and L147 are high yielding and have a good combining ability for yield. Only clone L93 looks less susceptible.

Table 2: CWD infection levels in "Parc à Bois", Yangambi, January 2000 (Planted 1964)
Numbers of plants infected/dead per row of 10 trees, deaths being attributed to CWD

N°	CLONE	Blocks						Infection	
		I	II	III	IV	V	VI	Total	%
1	L251	5	3	9	3	3	8	33	55%
2	L215	8	2	3	5	6	6	30	50%
3	SA158	5	4	2	1	3	6	21	35%
4	L147	8	7	4	5	7	4	35	58%
5	L 93	2	2	2	0	2	2	10	17%

3.2 Trial Field "Local Adaptation of Petit Kouillou variety", Yangambi

This trial was established in 1989. It compares variety "Petit Kouillou" (from Bas Cong with seeds from clones L251, L147, LAF159, and S21, in rows of 12 plants, of which 10 are assessed for yields. Results in Table 3 show that Petit Kouillou is susceptible but is less attacked than other clones.

Table 3: CWD infection levels in "Local Adaptation of Petit Kouillou variety", Yangambi, January 2000 (Planted 1989)
Numbers of plants infected/dead per row of 10 trees, deaths being attributed to CWD

N°	Progeny	Blocks						Infection	
		I	II	III	IV	V	VI	Total	%
1	Petit Kouillou	3	2	-	2	-	1	8	13%
2	S21	2	2	-	3	2	1	10	17%
3	LAF 159	2	1	2	6	5	1	17	28%
4	L 147	3	2	2	3	1	-	11	18%
5	L 251	-	1	1	6	5	-	11	18%

3.3 Robusta x Petit Kouillou hybrids

The purpose of this observation trial was to improve the bean size of Petit Kouillou. The various crosses made did not produce enough seeds. The controls were clones L251 and L147. Results of CWD attack are presented in Table 4. In this trial only Hybrid L93 x S1 is affected, at a very low level. Unlike their parents in the clone trial, the progenies of Clones L147 and L251 have a very low level of attacks. Other hybrids established in this trial do not have enough plants to make any conclusion at this stage.

Table 4: CWD infection in the Robusta x Petit Kouillou hybrid trial, Yangambi, January 2000 (planted 1991)
Numbers of plants infected/dead, deaths being attributed to CWD

Clone / Progeny	Total planted	Infected plants
Clone L 251	44	7
Clone L 147	44	1
Hybrid L 93 x S1	32	3
Hybrid L 93 x S23	22	0
Hybrid SA 158 x S9	12	0
Hybrid L36 x S1	11	0
Hybrid SA 158 x S23	6	1
Hybrid L 93 x S 19	5	0
Hybrid L 36 x S19	2	0
Hybrid L 215 x S1	1	0
Hybrid L36 x S23	4	0
Hybrid L93 x S9	6	0

Seeds collection and dispatch to partners: perspectives

For the Robusta variety 7 clones are currently released to the farmers, namely L36, L48, L93, L147, L215, L251, and SA158. This material is available and well identified at Yangambi Research Station, which is just becoming reachable for us. They will be regularly harvested for dispatch to the partners.

For the Bas Congo region the list of recommended clones is different (Petit Kouillou variety), namely: LAF93, LAF159, S9, S19, S23.

Kiyaka Research Station is situated 5°16' S, 18°57' E at 750 m altitude, and at more than 560 km from Kinshasa. One of its goals is to distribute planting materials adapted to the region. Two Robusta blocks are established from materials selected at Yangambi. High yielding individual trees have been mass selected, and are being used as a source of seeds for screening.

Seeds will be regularly collected at Yangambi and Kiyaka, and at Luki, for Robusta and Kouillou respectively.

WP4. DISEASE: EPIDEMIOLOGY

The coffee regions can be split into 3 main sectors:
- Southern: Bandudu, Bas Congo, Western and Eastern Kasai provinces
- Northeast: North Kivu provinces (Beni and Butembo)
- Northern: Eastern Equator provinces (Kisangani and Yangambi)

For practical and financial reasons, and in relation with the state of war that prevailed up to now in the other regions, the Southern region was visited first.

Coffee farms representative of the region were sampled as follows:

a) 12 locations per site, representing one or more groups of villages
b) In each location the assessment is done for at least 5 small farms and 5 large plantations for each group of villages.
c) In each farm at least 30 trees were surveys for small farms, and more than 30 for bigger farms depending on their size.

The criterion used to identify the sick trees was the presence of symptoms characteristic of the disease i.e. blackening of the bark associated with, at collar level, brownish wood under the bark with presence of black strips.

4.2 Characteristics of the Southern sector

4.2.1 Localisation

The Southern Coffee Sector is made of 4 provinces (autonomous administrative divisions)

The Bas Congo Province is situated 4-6° S and 12 – 16° E. Its area is 53,947 km². Administratively it is made of 3 districts divided into 3 territories divided into Sectors (Figure 1).

Bandundu Province lays between 8 and 10 ° S, 16° and 21° E. It covers 298,658 km². It is divided into 4 districts and 2 towns, Bandundu and Kikwit (Figure 2).

Kasai, in the centre of the country (330,077 km²), is made of 2 provinces:
- Western Kasai Province (156,967 km², 2°28'S – 8°3'S and 19°40'E – 23°41'E, altitude 580-1000 m, Figure 3)
- Eastern Kasai Province (173,110 km², 1°43'S – 8° S, 21°47'E – 26°17'E, elevation 450-900 m, figure 4)

Both Provinces cover 6.7 and 7% respectively of the total country area.

4.2.2 Climate

Coffee is grown in regions with conducive eco-climatic conditions.

Bas-Congo, being South of the Equator, is under constant influence of South Eastern winds. Dominant winds are influenced by the Benguela cold sea streams. The province has a Sudanese tropical climate with 5 dry months (May to mid-October) and two heavy rainy periods (March-April, and November)

Bandundu and Kasai have two distinct climatic types:

- Equatorial type Af (Köppen's classification) and the North: absence of regular dry season (North East of Lake Maï-Ndombe i.e. Inongo, Kiri, and Oshwe-North territories), Dekese in Western Kasai, and Lomela in Eastern Kasai.
- Sudanese type (Köppen's "Aw") with one rain season and one dry season; the latter increases when one gets further away from the Equator.

Average temperatures are close to optimal for coffee, without marked fluctuations.

Generally speaking rainfall averages 1,500 – 1,800 mm per year in the Southern coffee sector. In Bas-Congo short but high rainfalls reach an annual total of 900-1500 mm. The average for Luluka is 1,360 mm with a 200 mm peak in January.

In Bandundu the highest annual rainfall averages are reached in the zones with Af type climate (500 to 2,000 mm, average 1,800 mm), and the lowest in the South (800 to 1,500 mm/year).

In Kasai average rainfall gets lower from North to South (2,000 to 1,500 mm).

It is assumed that Coffee thrives best in climates with periods both wet and dry, the latter corresponding to vegetative rest before the main flowering.

The required temperature (20-25°C average with minimum 15°C and maximum 30°C) are obtained in these regions.

In Bas Congo the annual mean is around 25°C (nights 23°C, days 34_38°C). In the dry season the average is 22°C (17°C and 23°C respectively night and day). The amplitude of differences is not above 10°C.

Records at Kiyaka indicate for Bandundu annual means around 25°C, with mean maxima of 28°C, and mean minima of 20.3°C.

In Kasai the average is 24-25°C in the North and 22.5°C in the South. The temperature lowers in July for the whole region, but the variation over the year is not much.

4.2.3 Soils

Although Coffee is not very demanding in terms of soils it is known that it may exhaust the soil.

Bas-Congo soils are clayish-sandy to clayish; they belong to the group ferralsoils derived from basic rock.

Bandundu has sandy soils in savannah zones, clayish-sandy soils in forest valleys (with good structure and favourable water balance), and poor sandy soils on plateaux.

Most soils in Kasai belong to kaolisols. In Western Kasai there are some ferralsoils (1,000 – 1,500 m altitude), orthotypes ferralsoils, washed ferralsoils, and areno-feeralsoils (500 – 1000 m), and hydro-ferralsoils on lower plateaux (360 – 380 m). In Eastern Kasai one can find very acidic and washed arenasoils, but North east has ferrisoils with a god structure, and good mineral reserves. In the South the soils belong to ferralsoils.

4.2.4 Coffee Zones, and Coffee farming systems

4.2.4.1 Zones

In Bas-Congo, most coffee zones are situated in the Bas-Fleuve District (Lukula and Tshela zones, North and West of Seke-Banza) on a clayish-sandy to clayish-peat forest soil, deep, draining, with a good structure, and with a good water balance, that are conducive to coffee growing. Relatively well covered by forest or by re-growth these soils are not endangered by erosion. The climate is hot and wet with favourable rainfall.
In Bandundu Province most coffee farms are in forest valleys, in forests or in forest galleries located between large, navigable rivers. Although the conditions are most conducive to coffee growing, the steep slopes may present a risk of degradation through erosion.
In Kasai, coffee is established in salty forest zones where the suitability for coffee depends on humus contents.

4.2.4.2 Cultural systems

Two types of plantations can be found in DRC.

- Village smallholdings, mainly gardens close to the houses or plants scattered. The average area is .8 to 1.3 ha.
- Large agro-industrial units that belong to local or foreign companies or individuals. The areas are above 50ha.

The part of smallholders in the country's production is about 70%.
In Bas-Congo the average area per farm is 1 ha except for the Kakongo community where small farms can easily reach 4 ha. In Mweka territory (Western Kasai) most farmers have access to grouped coffee garden that average 2-3 ha each. In Bandundu, the areas are small.

During our visits we could see that many industrial farms were being abandoned mainly due to economic difficulties and to the lack of manpower. In the case of Luebo for instance the farm was converted to other crops.

4.2.4.3 Coffee Planting materials used

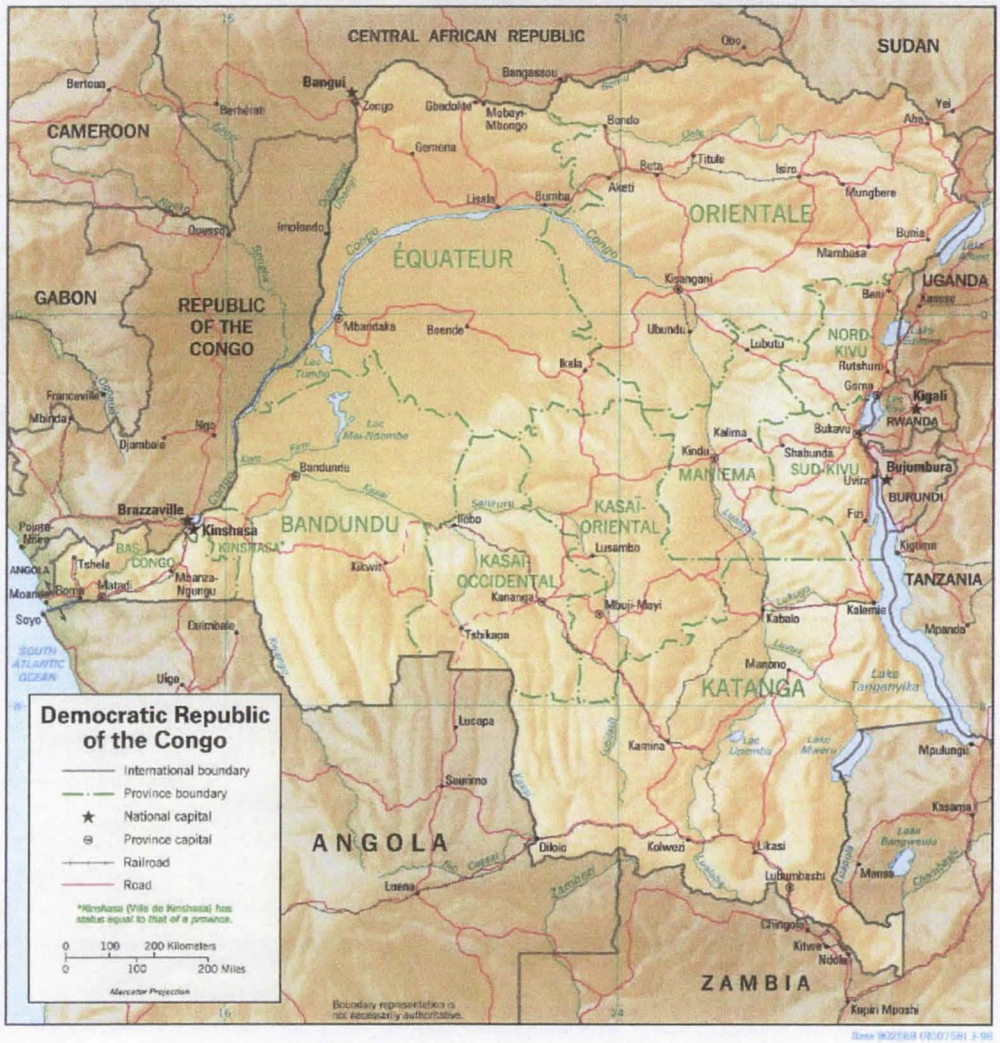
Most small or big farmers do not know what type of planting material is established on their farm. It is quite difficult to precisely determine the variety.

In the visited regions there was not multiplication centre, which means that most coffee trees were obtained from seeds derived either indirectly from selected clones or from other plantations, thus not from improved varieties.
In Bas-Congo the variety Petit Kouillou is widely used. It is made of a mixture of 5 selected clones, namely L93, L159, S9, S19, and S23. INERA Research Station at Luki provided this material to the farmers.
In Bandundu and Kasai, the variety grown is Robusta, selected and distributed by Bena Longo and Mukumari (Sankuru) INERA Stations. We were told that the initial material came from Yangambi, and comprised the standard clone material i.e. clones L36, L48, L93, L147, L215, L251, and SA158.

Kiyaka Research Station has 2 blocks (3 ha) established in 1954 and 1963. These Blocks are currently maintained, and will be used as a source for plant materials for our studies.

4.2.4.4 Situation of Coffee Wilt Disease

No case of the disease could be found in any of the site visited. The mortality rate is very low (less than 10%).
Cases reported by farmers or by other agriculturists are obviously wilts in connection with root rots.
The lack of basic phytotechnical practices and of regular phytosanitary controls may have favoured the expansion of the disease if a spot had appeared. Luckily it did not happen.
Attacks of cercosporiose, of root rots and of Leaf Rust have been noticed here and there, without a real economical impact.



Administrative map of RDC

CABI

INTERNATIONAL SCIENTIFIC COOPERATION PROJECT (INCO)

Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease

First Annual Report (November 2001 to October 2002)

CABI Bioscience (CAB International)
UK Centre, Bakeham Lane, Egham,
Surrey, TW20 9TY, UK

INCO-DEV COFFEE WILT PROJECT

(Contract no. ICA4-CT-2001-10006)

Work Package 1: Pathogen Diversity
Annual Report
October 2002

Dr M. A. Rutherford
CABI Bioscience, Egham, UK

Contents

1. Project partners

CABI Bioscience (CABI), United Kingdom (leading Work Package 1)
Centre de Cooperation Internationale en Recherche Agronomique pour Le Developpement (CIRAD), France
Coffee Research Institute (CORI), Uganda
University of Kinshasa (UoK), Democratic Republic of Congo
University of Louvain (UoL), Belgium

2. Overall objective

To improve knowledge of the coffee wilt pathogen, *Fusarium xylarioides*, with respect to genetic diversity and variation in aggressiveness.

3. Specific objectives

To establish a collection of isolates of *F. xylarioides* from various regions and examine the level of diversity among isolates, across geographical locations and over time, using a range of mycological and molecular techniques to determine if a new strain of the pathogen has emerged and become prevalent. The possible role of the sexual phase of the life cycle of *F. xylarioides* in the evolution of any genetic diversity will be evaluated as part of this work. Isolate aggressiveness towards coffee hosts will also be evaluated to determine if variability exists, in what form and whether it is changing and to facilitate selection of isolates representative of the range of variability for use in routine screening tests.

4. Tasks

Task 1: Collection of anamorph and teleomorph forms of the fungus on various parts of the trees, possibly on alternative hosts, in affected regions. Maintenance and dispatch to European labs. (CORI, UoK with participation of CIRAD, CABI)

Task 2: Identification, storage, and exchange of isolates. Use of a designated facility to ensure the successful maintenance of the pathogen strains. All data used to characterise the strains to be maintained on a database (CABI).

Task 3: Evaluation of the variability in isolate aggressiveness using standard inoculation tests (Work Package 1). (CIRAD, UoL, CORI, UoK)

Task 4: Description of the fungal life cycle, asexual and sexual phases. (CIRAD, CORI, UoK)

Task 5: Evaluation of the genetic diversity using a range of techniques including PCR and microsatellites. (CIRAD, CABI)

Task 6: Combine the results of all above tasks in order to propose explanations on the evolution of the fungus. (CABI)

5. Progress

5.1 Task 1: Collection of anamorph and teleomorph forms of the fungus on various parts of the trees, possibly on alternative hosts, in affected regions. Maintenance and dispatch to European labs. (CORI, UoK with participation of CIRAD, CABI)

Plant material, namely pieces of stem tissue and branches, has been collected from coffee trees exhibiting symptoms of wilt disease. This has been possible through visits made to coffee growing areas (including established coffee germplasm sites) specifically for this purpose and during surveys undertaken as part of associated coffee wilt disease projects funded by the European Union (EU) and the Common Fund for Commodities (CFC). In Uganda, branches and stem pieces (15-20 cm in length) were collected from farmers' fields (for details see Annex 3) and also from 10 *Coffea canephora* (robusta coffee) and *C. excelsa* genotypes held at the hybrid trial sites at Kituza and at the germplasm collection at Kawanda Agricultural Research Institute rerspectively. No collections have been made in the Democratic Republic of Congo (DRC) as yet. However, this will be possible through epidemiological surveys in Northern and North Eastern DRC planned for the near future.

To date, isolations of *F. xylarioides* from plant material have been made in the country of origin of the material, and within seven days of collection, and isolates subsequently forwarded to CABI and other European partners. However, where appropriate and particularly where perithecia are observed, wood samples will be forwarded to UoL for investigations of the sexual stage of the fungus and isolation of *F. xylarioides*. Where possible, the collection, isolation and purification protocol developed during the inaugural project workshop held in Kampala (February 2002) was followed (see Annex 1).

5.2 Task 2: Identification, storage, and exchange of isolates. Use a designed facility to ensure the successful maintenance of the pathogen strains. All data used to characterise the strains to be maintained on a database (CABI).

To date, more than 150 isolates of *F. xylarioides* have been made available for the work package research, of which more than 100 are now being held at CABI Bioscience (CABI). Those held at CABI have either been donated from culture collections held elsewhere, isolated from coffee plant material as part of previous wilt investigations undertaken at CABI or freshly isolated from plant material under this prject and as described under task 1 above. Unfortunately, attempts to isolate *F. xylarioides* from stem samples collected in the North Eastern (Isiro, Province Orientale) and Eastern (Beni-Butembo, North Kivu) regions of DRC between 1995 and 1998 and held at UoK were unsuccessful. However, two *F. xylarioides* isolates from Isiro and Beni are available and will be dispatched to partners.

All isolates received at CABI have already been, or will be, purified by single sporing prior to analysis proceeding and assigned a unique accession number (or 'IMI' number), e'g' IMI23456, to be quoted in all correspondence and held on the information database under development at CABI (see below). Allocation of an accession number will also apply to *F. xylarioides* isolates obtained from plant specimens received at CABI. The identification of all isolates received at CABI is also confirmed as *F. xylarioides* by CABI mycologists prior to other investigations proceeding. CABI has, and will continue to, redistribute isolates as appropriate to other European partners to facilitate their research but, given quarantine restrictions and the potential threat to coffee production. CABI will not provide isolates to partners in Africa other than those whose return to their country of origin is permitted by the agricultural authorities.

All purified (single spored) isolates of *F. xylarioides* are being maintained on Synthetic Nutrient Agar (SNA) medium for short-term storage at CABI. Isolates will also be freeze dried and/or placed under liquid nitrogen and deposited in the CABI Genetic Resources Collection for safe, longer term storage.

Development of an electronic database, based on 'Access' software, to store key information relating to the *F. xylarioides* isolates obtained for the work package research has been initiated. Ultimately all baseline information on each isolate, such as geographic origin and host type, type of analyses undertaken and results of key analyses will be inputted and may be accessed by all partners involved in the work package.

5.3 Task 3: Evaluation of the variability in isolate aggressiveness using standard inoculation tests. (CIRAD, UoL, CORI, UoK)
Six *C. canephora* (robusta) clones, 1s/2, 1s/3, 1s/6, 223/32, 257/35 and 258/24(0), were recommended by the Ugandan government as replacements for the existing planting material in Uganda prior to the coffee wilt outbreak in the early 1990s. Although mass multiplication of planting material of these clones commenced, pathogenicity testing undertaken in 1997 (involving artificial inoculation of pot grown coffee plantlets with *F. xylarioides* under screenhouse conditions) showed that all six clones developed symptoms of the disease but that the level of susceptibility varied. Similar reactions were observed under field conditions. This research is being followed-up as part of the current project. Plantlets of these clones currently being raised in the nursery at Kituza will be inoculated with *F. xylarioides* by various techniques, including stem injection and root dipping, to determine which method is most effective and consistent with regard to symptom development. These trials will commence in November 2002. The selected inoculation technique will subsequently be used as a 'ring test' by project partners involved in resistance screening trials, due to commence in approximately three months time when plantlets are 5-6 months old. Plantlets of the six recommended clones are also currently being produced for this work, although other clonal types may also be included if considered appropriate. To avoid transfer of plant material between African coffee producing countries, pathogenicity testing with isolates originating from Ugandan coffee will be undertaken at CORI, CIRAD and UoL, those from DRC coffee at UoK, CIRAD and UoL.

5.4 Task 4: Description of the fungal life cycle, asexual and sexual phases. (CIRAD, CORI, UoK)
Activities relating to task 4 will commence in 2003. This work will focus on development of a model system to investigate the sexual cycle of *F. xylarioides*, analysis of character segregation in the sexual cycle and an assessment of the likelihood of isolates capable of overcoming arabusta clones arising through the sexual cycle. Based on VCG tester strains developed at CABI, vegetative compatibility between progenies of isolate crosses produced in mating tests will also be assessed.

5.5 Task 5: Evaluation of the genetic diversity using a range of techniques including PCR and microsatellites. (CIRAD, CABI)
Previous investigations of molecular variability within *F. xylarioides* isolates obtained from wilted robusta coffee trees in Uganda and DRC and wilted arabica trees in Ethiopia were undertaken at CABI in 1997/8. The results of this work, which was based on genomic fingerprinting using arbitrary PCR primers and digestion of the ribosomal DNA Intergenic Spacer (IGS) region, showed that there was very little heterogeneity within the group of isolates obtained from robusta in Uganda and DRC. However, this group was markedly different from the isolates obtained from arabica coffee in Ethiopia, suggesting that host specialisation may be operating.

This earlier work was undertaken on a limited range of isolates from these countries. Research being undertaken at CABI as part of the current project will use these and other, more recently developed, molecular techniques to analyse variability among isolates from a broader range of range of origins. By including representative isolates from previous work, it will be possible to assess whether the level of diversity has changed geographically or over time, to determine the current level of host specificity between and within coffee species and to determine whether any new strains may have emerged. This work will be supported by a conventional genetic approach to analysis of variability, whereby the presence of vegetative compatibility groups (VCG) among isolates of *F. xylarioides* will be determined.

For preliminary analysis of molecular variability and VCG, a representative subgroup of 15 isolates from Uganda and DRC (see Annex 2) has been selected. Extraction of DNA from these isolates, using a modified CTAB technique developed by Cubero *et al.* (1999)*, has yielded high quality DNA from each isolate. All DNA samples were amplifiable by PCR. Analysis of molecular variability using variable number tandem repeat (VNTR) primers and digestion of the rDNA IGS has commenced. Results of initial tests showed that amplification of DNA by VNTR primers RY (CAG^5] and ERIC 2 (enterobacterial repetitive intergenic consensus) primers each gave rise to almost identical fingerprint profiles for all strains. However, when amplified using five separate inter-simple-sequence-repeat-anchored (ISSR) PCR primers, AAC, ACA, CCA, CGA & TGT, primer CGA revealed no varition between the strains but each of the remaining primers demonstrated some level of intraspecific variability. ISSR is considered to be a useful genotyping tool for assessing variability across the entire genome (Taylor et al., 1999*). Data from this preliminary work will undergo full analysis shortly. Follow-on studies, now being initiated, will involve further amplification by ISSR primers in addition to single-enzyme (agarose gel-based) and dual-enzyme (acrylamide gel-based) amplified fragment length polymorphism (AFLP) analyses.

Cubero et al. (1999).Pl. Syst. Evol. **216: 243-249*
*Taylor et al. (1999). Ann. Rev. Phytopath. **37**, 197-246*

The molecular analyses are being supported by a conventional genetic approach involving assessment of vegetative compatibility between isolates. Mutant forms of the wild type isolates, that are unable to utilise nitrate and are required for subsequent pairing and vegetative compatibility group (VCG) identification, are also being generated and typed at CABI at present. Similar studies will be undertaken on *F. xylarioides* isolates originating from DRC during a training of a Congolese scientist on attachment to the University of Louvain.

Results of the molecular and VCG analyses will ultimately be compared to those for other approaches, e.g. specificity and aggressiveness of isolate to different coffee types, to determine if any correlation exists and, where possible, to identify specific markers for particular traits.

5.6 Task 6: Combine the results of all above tasks in order to propose explanations on the evolution of the fungus. (CABI)

Key findings from the various research activities being undertaken by the work package partners is being collated by CABI. Collation and analysis of data and interpretation of findings is being facilitated by establishment of the electronic database to which data is as and when available. As the work package research has only recently been initiated it much too early to try to draw any conclusions from the activities undertaken to date. However, this report summarises the activities undertaken to date and provides an overview of the progress being made.

6. Outline plans for next year

- Collection of symptomatic coffee plant material, from which *F. xylarioides* will be isolated, will continue across the coffee production areas of Uganda and DRC and isolates forwarded to CABI. Acquisition of isolates from other culture collections, and exchange of isolates between research partners (as appropriate) will also continue.
- Studies to elucidate the role of the teleomorph (*Gibberella xylarioide*), including monitoring of periothecia and conidia production on selected wilted trees in coffee producing areas and analyses of ascospore genetics, will be initiated. Pieces of wood (and branches if appropriate) bearing perithecia will be sent to UoL to facilitate similar research.
- A procedure suitable for inoculating coffee plants with *F. xylarioides* and eliciting wilt symptoms will be identified in the early part of the year and will be used in subsequent host plant inoculations to determine the relative aggressiveness of isolates.
- The database pertaining to the various studies being undertaken under work package 1 will continue to be updated at CABI and routinely disseminated to other project partners. CABI will continue to oversee, and provide progress reports as required on, the various work package activities undertaken throughout the year.

7. Publications and papers

Rutherford, M A (2002). Development of a long-term strategy based on genetic resistance and agro-ecological approaches against Coffee Wilt Disease. Annual Progress Report (Nov. 2001-Oct. 2002) for Work Package 1 (Pathogen Diversity), INCO-DEV contract ICA4-CT-2001-10006. Oct. 2002.

Annex 1

PROTOCOLS FOR COLLECTION AND HANDLING OF PLANT MATERIAL AND ISOLATION OF *FUSARIUM XYLARIOIDES*

Collection of plant samples (to be applied where appropriate and practical)

Note: unless stated otherwise, plant samples will be removed from the main trunk of the tree and where discoloration of the underlying wood is visible.

Country level: Collections of plant material should initially be made from as wide a geographical area as possible. It is therefore suggested that a total of 50 samples be collected at country level, each from one tree at 50 locations.

Area level: Three areas will be selected from the main coffee growing areas that have serious coffee wilt disease problems and that are geographically separated. A sub-district is suggested to be the area chosen and within that two samples, one from each of two trees, should be collected from each of 25 farms.

Farm level. Collections to be made from every diseased tree at three farms at each of the three sub-districts.

Clone level. Collections made from 20 trees in the clonal trial at CORI and from the clonal trials at Yangambi in DRC.

Plant Level. Collections made from two trees from each of three farms. The farms will be the same as those visited in the country and area collections but each of the two trees will be sampled extensively i.e. isolations made from all plant parts including roots.

Handling plant samples

Always use paper bags. Specimens placed in plastic bags are liable to sweat and growth of contaminants will be encouraged.

Try to remove clean samples and wrap individually in newspaper.

Several plant samples from the same tree may be placed in one bag provided they are dry and wrapped individually in newspaper.

Samples must be clearly labelled with respect to farm, location, tree, part of tree etc. Where possible, GPS data should be taken for accurate siting of location.

Isolation methods

Ensure that the sample of plant material is clean. If not, wash under running tap water.

Once clean and rehydrated in water, pieces of wood or bark should be surface sterilized for 1–2 mins in 1 % sodium hypochlorite for thick barked specimens or for 1-3 mins in 30% hydrogen peroxide for thinner barked specimens.

Rinse specimens in sterile distilled water.

Using aseptic techniques, scrape away the outer bark and remove underlying discolored areas of wood with a sharp scalpel.

Plate small pieces of wood onto 3% water agar plates and incubate at 20-25C for 2-3 days.

Single spore (SS) cultures should be prepared from young colonies emerging from plant by streaking out microconidia onto 3% water agar and incubating overnight to allow germination.

Under a X40 objective single, germinating spores should be marked and picked off with the hypha(e) intact using a tungsten wire needle or by cutting out an agar block. These should be transferred to fresh SNA agar plates, one per plate.

Once single spore cultures are obtained, duplicate cultures should be prepared. One culture should be to to CABI where it will be stored and analysed. The other culture should be retained by the relevant partner.

SS isolates should be maintained and stored over the short term on SNA medium. Low nutrition medium such as SNA reduces the morphological degeneration of isolates, and possibly stabilizes other traits such as metabolite production and pathogenicity. Cultures should not be kept on rich, complex agar media such as potato sucrose agar (PSA) or potato dextrose (PDA) for prolonged periods otherwise degeneration is likely to occur rapidly.

Annex 2

REPRESENTATIVE SET OF *FUSARIUM XYLARIOIDES* ISOLATES SELECTED FOR PRELIMINARY ANALYSIS OF MOLECULAR VARIABILITY AND VCG TESTING AT CABI

Isolate no.	Other isolate no.s	Host plant species	Country of origin	Locality	Single spored	DNA extracted
W5106		*C. canephora* (robusta)	DRC	Rutchuru area, North Kivu	Yes	Yes
W5272b	E	*C. canephora* (robusta)	DRC	Nzuki plantation, Capaco	Yes	Yes
W5280b	A	*C. canephora* (robusta)	DRC	Seeurs plantation, Orantes	Yes	Yes
W5263c	B	*C. canephora* (robusta)	DRC	Umbru plantation, Mandimbo	Yes	Yes
W5267a	C	*C. canephora* (robusta)	DRC	Apoyo village (Mr Mbodupa)	Yes	Yes
W5432a		*C. canephora* (robusta)	Uganda	Milambi village	Yes	Yes
W5433a		*C. canephora* (robusta)	Uganda	-	Yes	Yes
W5440a		*C. canephora* (robusta)	Uganda	Kamirundi village (Mr Kakorato)	Yes	Yes
W5448a		*C. canephora* (robusta)	Uganda	Wabilongo village (Mr Nswani Ibrahim)	Yes	Yes
W5554a		*C. canephora* (robusta)	Uganda	-	Yes	Yes
H1		*C. canephora* (robusta)	Uganda	Mukono district	Yes	Yes
M		*C. canephora* (robusta)	Uganda	Rukungiri district	Yes	Yes
R		*C. canephora* (robusta)	Uganda	Kyenjojo district	Yes	Yes
S		*C. canephora* (robusta)	Uganda	Kibale district	Yes	Yes
X		*C. canephora* (robusta)	Uganda	Bundibugyo district	Yes	Yes

Annex 3

GEOGRAPHIC ORIGIN OF *F. XYLARIOIDES* ISOLATES OBTAINED FROM ROBUSTA COFFEE GROWING AREAS OF UGANDA AND RECEIVED AT CABI

Isolate code	Subcounty	County	District
F1	Kakindu	Busuzu	Mubende
F2	Kasambya	Buwekula	Mubende
H1	Kasawo	Kasawo	Mukono
L	Kibibi	Butambala	Mpigi
M	Kebisoni	Rubabo	Rukungiri
O	Busana	Busana	Kayunga
P	Wakiso	Wakiso	Wakiso
Q	Bukulula	Kalungu	Masaka
R	Butiti	Mwenge	Kyenjojo
S	Masaka	Bugangaizi	Kibale
T	Kalagala	Bamunanika	Luwero
U	Ntwetwe	-	Kiboga
V	-	-	Kalangala
W	Muwanga	Kiboga	Kiboga
X	Bubandi	Bwamba	Bundibugyo
15	Kakindu	Busunssu	Mubende
23	Kiboga	Kiboga	Kiboge
25	Bukuya	Kassanda	Mubende
34	-	-	-
39	Kakindo	Bugangaizi	Kibale
52	-	-	-
67	-	-	-
72	-	-	Kibale

ANNEXES

Meeting reports

The report of the inaugural workshop (Kampala 4-7 March 2002) has been edited in the first intermediate report.

Relevant information

Copy of the MTA enclosed in annex

COFFEE RESEARCH INSTITUTE (CORI)

KITUZA

P.O. BOX 185, Mukono - Uganda
Tel: 256 77 700725 / 6
Fax: 256 77 250729
E-mail: cori@africaonline.co.ug

Our Ref: .....01.11.00.....

Your Ref: .....

October 30, 2002

Dr. Daniel Bieysse
CIRAD – AMIS
PHYTROP TA 40-02
Avenue Agropolis
34398 Montpellier Cedex 5
FRANCE

Tel: 33 (0) 4 67 61 44 68

Dear Dr. Daniel

MATERIAL TRANSFER AGREEMENT (MTA)

Thank you very much for being understanding and patient while trying to get this MTA revised and signed. I am glad that at last all has worked out well.

Please find enclosed two (2) original signed copies for Dr. Mourichon and yours. Lets look forward for further cooperation and collaboration including the INCO-COWIDI work.

Assistance rendered is highly appreciated.

Yours sincerely

Dr. Denis T. Kyetere
Director of Research

Encl. (2)

AGREEMENT

MATERIAL TRANSFER AGREEMENT

Preamble

The Material Transfer Agreement (MTA) will govern the exchange of selected biological material between:

i) Coffee Research Institute (CORI)
P.O. BOX 185
Mukono, Uganda

CORI is herein represented by The National Agricultural Research Organisation (NARO), P.O. BOX 295, Entebbe, Uganda, which represents the public-sector national agricultural research system. NARO is hereinafter known as the "Provider".

and

ii) Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), 42 Rue Scheffer, 75116 Paris Cedex, France or hereinafter known as the "Recipient" (R) of whom the main collaborator will be Dr. Daniel Bieysse.

Basis

This MTA is based on a collaborative Project "Development of a long term strategy based on genetic resistance and agroecological approaches against coffee wilt disease in Africa". Project Acronym, COWIDI, Project Proposal No. ICA4-2000-10312 under INCO-DEV.

Scope

In this Project, a student from Uganda Mr. Pascal Musoli, will carry out part of his Ph.D. research project at CIRAD. He will be evaluating seedlings and cuttings of the biological material. It is understood that all exchange of biological material will be done strictly according to the principles in the Convention of Biological Diversity.

The Provider and Recipient therefore agree as follows:

1. The Biological Material covered by this Agreement is known as *Coffea spp.* and for the purpose of this Agreement this Biological Material includes seeds and cuttings.

2. The Biological Material is being supplied to Dr D. BIEYSSE solely to be used for research in the INCO-DEV Project COWIDI within his own immediate laboratory in CIRAD Montpellier France.

3. NARO is willing to supply a sample of the Biological Material subject to the following conditions:

(i) It is used for strictly non-commercial purposes and will not be used by [R] for, nor supplied to any other parties for commercial purposes, even if those purposes are being pursued in the same laboratory.

(ii) The Biological Material remains the property of NARO at all times. It will not be removed at any time from the above [R] address given above nor provided to any other person or parties, except for Organisations involved in the same INCO COWIDI Project, namely Université Catholique de Louvain (UCL) and Faculté d'Agronomie de l'Université de Kinshasa (FACAGRO/UNIKIN), subject to specific authorisation by NARO.

(iii) The Biological Material is made available for the purpose of the Work, stated in the scope, and will not be used for any other purpose without the express, written consent of NARO. [R] will handle the Biological Material in compliance with Uganda and international laws, regulations and guidelines which may be applicable at the time of use.

(iv) The Biological Material will be handled only by those with sufficient skill, knowledge, experience and ability applicable to the Biological Material. NARO and its employees cannot be liable for any loss, damage, claim or any other liability which may arise from the use of the Biological Material.

(v) The Biological Material is experimental in nature and is provided without any warranty or guarantee with respect to its performance or fitness for any particular purpose or to the completeness and accuracy of any information related to the Biological Material supplied by NARO.

(vi) NARO can provide neither assurance nor warranty that the Biological Material or its use is free from patent and other intellectual property rights.

(vii) In the event that the Recipient makes or observes any new discovery, improvement or invention ("Invention") relating to the Biological Material, whether patentable or not, the Recipient will act to ensure that the protection of any rights to the Invention is not destroyed nor endangered by way of disclosure or any other route and shall bring this to the immediate attention of NARO. NARO will act to ensure that the Invention is appropriately protected. The Recipient CIRAD and the partners FACAGRO/UNIKIN and UCL will not make any patent or secure other intellectual property rights without the express agreement of NARO. A further agreement will be sought to specify the share of any benefits from the exploitation of Invention. NARO will, at all times, retain the right to use any Invention.

(viii) The Recipient will fully acknowledge the source of the Biological Material in any publication arising from studies in which the Biological Material is referred to and will notify NARO of any such publication under preparation, date of submission and supply a copy of the submitted draft and final publication. This will, however, not interfere with the student's rights.

(ix) At any time requested by NARO or in the event that the Recipient fails to comply with the conditions and provisions of this Agreement, the Recipient shall immediately return the Biological Material to NARO and destroy any copies of the Biological Material which may have been made in the course of the Work.

(x) The duration of this Agreement is up to the end of completion of the research specified under INCO-COWIDI.

(xi) This Agreement shall be governed by the laws of Uganda.

(xii) Both parties may amend this agreement at the instance of either party and in agreement with one another. Any such amendment will be annexed to this agreement and form part of it.

(xiii) Where a dispute arises concerning any matter related to this agreement it will be settled amicably by the two parties. If the two do not, they may jointly seek the good offices of, or request mediation by, a third party.

(xiv) This agreement will come into effect on the date of signature.

Agreed to:

Date October 15, 2002

Dr. Denis T Kyetere
Director of Research
Coffee Research Institute

Date 25/10/2002

Prof. Joseph K. Mukiibi
Director General
National Agricultural Research Organisation

DIRECTOR GENERAL
NATIONAL AGRICULTURAL
RESEARCH ORGANISATION (NARO)

For NARO

---ooOoo---

Date 9 10 2002

Dr. Xavier Mourichon
Director of CIRAD Plant Protection

Date 9/10/2002

Dr. Daniel Bieysse
Coordinator INCO Project COWIDI

For the Recipient

Annexe 2

Contract number : ICA4-CT2001-10006 Partner CIRAD Year1

Data sheet
for annual report

(to be compiled by **the co-ordinator** at 12-monthly intervals from start of contract. Figures to be up-dated **cumulatively** throughout project lifetime)

1. Dissemination activities

	Totals (cumulative)
Number of communications in conferences (published)	1
Number of communications in other media (internet, video,)	0
Number of publications in refereed journals (published)	0
Number of articles/books (published)	0
Number of other publications	0

2. Training

Number of PhDs	0
Number of MScs	1
Number of visiting scientists	0
Number of exchanges of scientists (stays longer than 3 months	0

3. Achieved results

Number of patent applications	0
Number of patents granted	*0*
Number of companies created	0
Number of new prototypes/products developed	0
Number of new tests/methods developed	1
Number of new norms/standards developed	0
Number of new softwares/codes developed	0
Number of production processes	0

4. Industrial aspects

Industrial contacts	yes	NO
Financial contribution by industry	yes	NO
Industrial partners : - Large	yes	NO
- SME [1]	yes	NO

S. Comments

Other achievements (use separate page if necessary)

Publications
COWIDI-Project. CIRAD-CORI-UNIKIN-CABI-UCL. Work-shop report "Development of a long term strategy based on genetic resistance and agroecological approaches against Coffee Wilt Disease in Africa. Kampala (Uganda) 4 to 7/03/2002.

Meetings
Oral presentation. BIEYSSE Daniel. The Coffee Wilt in Africa. Journées de Septembre. CIRAD- Montpellier (France). 28/08 to 6/09/2003

[1] Less than 500 employees.

Annexe 2

Contract number : IC.A4.-CT-2001-10006 Partner UCL Year 2002

Data sheet
for annual report

(to be compiled by **the co-ordinator** at 12-monthly intervals from start of contract. Figures to be up-dated **cumulatively** throughout project lifetime)

1. Dissemination activities

	Totals (cumulative)
Number of communications in conferences (published)	1
Number of communications in other media (internet, video,)	0
Number of publications in refereed journals (published)	0
Number of articles/books (published)	0
Number of other publications	1

2. Training

Number of PhDs	0
Number of MScs	0
Number of visiting scientists	0
Number of exchanges of scientists (stays longer than 3 months	0

3. Achieved results

Number of patent applications	0
Number of patents granted	0
Number of companies created	0
Number of new prototypes/products developed	0
Number of new tests/methods developed	0
Number of new norms/standards developed	0
Number of new softwares/codes developed	0
Number of production processes	0

4. Industrial aspects

Industrial contacts		no
Financial contribution by industry		no
Industrial partners : - Large	no	
- SME [1]		no

S. Comments

Other achievements (use separate page if necessary)

MARAITE H. 2002. The life cycle of *Gibberella xylarioides* Heim & Saccas. Proceedings of the First General Meeting of the COWIDI Project at Kampala, Uganda, 4-7 March 2002 .

MARAITE H. 2002. Instructions for "Monitoring of perithecia and conidia production of *Gibberella xylarioides* on naturally infected coffee trees". Proceedings of the First General Meeting of the COWIDI Project at Kampala, Uganda, 4-7 March 2002

[1] Less than 500 employe

Annexe 2

Contract number : ICA4.-CT-2001-10006 Partner UNIKIN Year 1

Data sheet
for annual report

(to be compiled by **the co-ordinator** at 12-monthly intervals from start of contract. Figures to be up-dated **cumulatively** throughout project lifetime)

1. Dissemination activities

	Totals (cumulative)
Number of communications in conferences (published)	3
Number of communications in other media (internet, video,)	1
Number of publications in refereed journals (published)	
Number of articles/books (published)	6
Number of other publications	1

2. Training

Number of PhDs	
Number of MScs	
Number of visiting scientists	
Number of exchanges of scientists (stays longer than 3 months	

3. Achieved results

Number of patent applications	
Number of patents granted	
Number of companies created	
Number of new prototypes/products developed	
Number of new tests/methods developed	
Number of new norms/standards developed	
Number of new softwares/codes developed	
Number of production processes	

4. Industrial aspects

Industrial contacts	yes	no
Financial contribution by industry	yes	no
Industrial partners : - Large	yes	no
- SME [1]	yes	no

S. Comments

Other achievements (use separate page if necessary)

[1] Less than 500 employees.

Annexe 2

Contract number : ICA4-CT2001-10006 Partner CABI Year1

Data sheet
for annual report

(to be compiled by **the co-ordinator** at 12-monthly intervals from start of contract. Figures to be up-dated **cumulatively** throughout project lifetime)

1. Dissemination activities

	Totals (cumulative)
Number of communications in conferences (published)	0
Number of communications in other media (internet, video,)	1 (internet)
Number of publications in refereed journals (published)	0
Number of articles/books (published)	0
Number of other publications	0

2. Training

Number of PhDs	0
Number of MScs	*0*
Number of visiting scientists	0
Number of exchanges of scientists (stays longer than 3 months	0

3. Achieved results

Number of patent applications	0
Number of patents granted	*0*
Number of companies created	0
Number of new prototypes/products developed	0
Number of new tests/methods developed	2
Number of new norms/standards developed	0
Number of new softwares/codes developed	0
Number of production processes	0

4. Industrial aspects

Industrial contacts	yes	NO
Financial contribution by industry	yes	NO
Industrial partners : - Large	yes	NO
- SME [1]	yes	NO

S. Comments

Other achievements (use separate page if necessary)

- Oral presentation entitled 'Isolation, collection and analysis of *Fusarium xylarioides* "presented at INCO-DEV Inaugural Workshop on INCO-DEV Coffee Wilt Project, Kampala, 3-7 March 2002 (proceedings published).

[1] Less than 500 employees.